

Report of the

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DESIGN, DEVELOPMENT, TEST, AND EVALUATION
OF AN
AUTOMATED ANALYTICAL ELECTROPHORESIS APPARATUS

for the period

17 May 1976 - 31 August 1977

NASA George C. Marshall Space Flight Center

Contract No. NAS 8-31948

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NEW TECHNOLOGY

The technical data contained in this report describe in some detail an apparatus that is considered to be a new and useful technology, and is therefore patentable. A patent disclosure will be filed by the University of Arizona in October 1977. At the same time a copy of the disclosure will be furnished to NASA Marshall Space Flight Center (New Technology Representative and Patent Representative). DD Form 882, "Report of Inventions and Subcontracts" will be forwarded.

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ABSTRACT

An automated analytical electrophoresis apparatus (AAEA) has been designed, developed, assembled, and preliminary tested by the Optical Sciences Center for the NASA George C. Marshall Space Flight Center for the period 17 May 1977 - 31 August 1977 under contract NAS 8-31948. The AAEA was demonstrated to be a feasible apparatus for automatically acquiring, displaying, and storing (and eventually analyzing) electrophoresis mobility data from living blood cells. The apparatus and the operation of its major assemblies are described in detail. It is concluded that good progress has been made to date and within the time and cost goals, toward the development of an automated analytical electrophoresis system. It is recommended that this program be continued to complete the second period (to 16 May 1978 vice 15 February 1978) for AAEA tests and evaluation utilizing live blood cells in order to determine and measure its operating parameters, as originally proposed. These must be established before undertaking clinical tests in a third, or subsequent period. Such a third period is strongly recommended. Equipment acquisition and fabrication costs to 31 August 1977 amounting to \$49,511 are detailed.

I. INTRODUCTION

This program stems from an unsolicited proposal by the Optical Sciences Center (OSC), University of Arizona, Tucson, AZ (OSC 75-42) dated April 22, 1975, to the NASA Marshall Space Flight Center (MSFC) for the development, fabrication, feasibility demonstration, and evaluation of an automated electrophoresis microscope system. The basic concept envisioned was an apparatus that would precisely and rapidly measure the velocities of various types of blood cells in an electrophoresis chamber and then compute and display their mobilities automatically. The need was established by the fact that available instrumentation was limited by the few blood samples that could be processed and with a technique that was tedious, complicated, and limited in precision. Available clinical electrophoresis instrumentation continues to suffer these limitations.

The above proposal resulted in NASA MSFC RFQ 8-1-6-EH-04835-AP13-1-1 dated 2 February 1976, which was responded to by OSC University of Arizona Proposal (OSC 75-042) of 1 March 1976. Subsequently, MSFC contract NAS 8-31948 was signed off by the University on 29 May 1976, retroactive to 17 May 1976, for a total estimated cost of \$83,780 for a 12-month initial period. The contractual scope of work for the first period and the specifications for the final apparatus were established as follows..

A. Scope of Work

Current space processing research activity in the field of separation technology is directed toward the development of equipment for the preparative

isolation of living biological cells. Compared to the wide-spread use of electrophoresis in protein separations, electrophoresis of cells is still in its infancy. This is largely due to the limitations of presently available instruments suitable for cell electrophoresis. There is a definite need for analytical instrumentation to determine the exact mobilities of living cells. The only technique presently available is microscope electrophoresis. This technique has remained essentially unchanged for the past fifty years. The technique consists of focusing a microscope on the interior of a suitable electrophoresis chamber, visually observing individual cells, and measuring their migration velocity in the electrical field by means of a stopwatch. This manual method of observation and measurement is extremely tedious, laborious, time-consuming, and inaccurate.

B. Objective

The objectives of this contract are to design and demonstrate the feasibility of an automated electrophoretic microscope system for determining and displaying the spectrum of mobilities of cells in a given population suspended in a compatible buffer and to establish its application as a tool for research, and as a clinical apparatus. The system will have suitable computer storage for subsequent data processing, and appropriate displays for visually monitoring the field of the microscope while acquiring data. An integral computer will evaluate all data over a given time interval and have the ability to correlate the data with other known parameters. This program will be divided into the following tasks:

"Task 1

Design all system components and interfaces. Submit to MSFC for approval

a complete drawing package and a list of all materials proposed for the system.

Task 2

After MSFC approval of design and all materials, the contractor shall build and test a breadboard model of the system to demonstrate the feasibility of the design.

Task 3

A complete functional test program shall be run in the presence of MSFC technical personnel. The system design performance goal will be to achieve a capability of measuring the velocities of 500 cells per sample in 10 min from a sample of 10^7 cells or less. Separate mobility distributions shall be collected at a rate of four per hour.

These tasks are to be completed by the contractor in one year after the award of the contract. Performance specifications for the completed automated analytical electrophoresis apparatus are listed below.

All of the specifications of the system cannot be met during the first period of performance but should nevertheless be considered in the initial design so that no major changes are required after the feasibility study."

C. Specifications

- "(1) The mobilities of approximately 500 cells should be obtained in 10 min from a sample of 10^7 cells or less. Separate mobility distributions should be able to be collected at a rate of four per hour.
- (2) Excluding problems associated with cell sedimentation the AAEA should be capable of collecting mobility data on nonpigmented

cells in the size range of 0.5 to 25 μm in diameter.

- (3) Each individual mobility determination should be accurate to $\pm 0.1\%$ over a mobility range of $0.2 \times 10^{-4} \text{ cm}^2\text{s}^{-1}\text{V}^{-1}$ to $8.0 \times 10^{-4} \text{ cm}^2\text{s}^{-1}\text{V}^{-1}$.
- (4) The AAEA must be capable of operating with suspending media of specific conductivity $< 0.021 \Omega^{-1}\text{cm}^{-1}$ over a temperature range of 25°C to 37°C and over a pH range of 2 to 11.
- (5) The chamber and electrodes shall be compatible with normal biological support media (containing proteins, carbohydrates, multivalent ions, etc.) in their operating configuration. Measurement conditions and chamber materials shall be such that cells undergoing measurement retain the same viability and surface properties as an appropriate control suspension not exposed to the instrument.
- (6) The AAEA should incorporate the capability to recognize and record other individual cell parameters besides electrophoretic mobility, as seem appropriate for the population under examination. In particular, the ability to distinguish between cells with and without a fluorescent label should be included in the AAEA.
- (7) As well as providing hard copies of electrophoretic mobility distributions, the AAEA should include the capability for detailed statistical analysis of the mobility data. These programs should provide:
 - (a) Descriptive statistics for the data.
 - (b) Assessments of the unimodality of the mobility distribution via the computation of distribution-free statistics for goodness of fit to known mobility distributions obtained from

calibration populations.

- (c) Test statistics to detect very small subpopulations of known mean and standard deviation, with errors of the first and second kind controlled to within known limits."

D. Personnel

Dr. Peter A. Bartels, an authority on automated cytology, data processing, and microscope instrumentation, is the Principal Investigator. He is a professor of microbiology and optical sciences at the University of Arizona and is also an adjunct professor of gynecology and obstetrics at the University of Chicago. His laboratory in the Microbiology Department is being used to house the assembled electrophoresis apparatus now being developed.

Co-Investigator, Dr. Milan Bier, is well known to NASA as an eminent investigator in preparative electrophoresis. At contract award time he was a research biophysicist at the U.S. Veteran's Hospital, Tucson, Arizona, with appointments as an adjunct professor in the Department of Aerospace and Mechanical Engineering and a visiting professor of biochemistry at the University of Arizona. In August 1977 he was appointed a full-time professor of mechanical engineering and microbiology at the University of Arizona. His laboratories are now located in the Departments of Microbiology and Electrical Engineering.

Dr. Geoffry V. F. Seaman of the University of Oregon is presently the program's only outside consultant, and he is regarded as a foremost expert in the field of analytical electrophoresis. He was the first to suggest to NASA the importance of the development of an automated device for the measurement of cell mobilities to meet the requirement for electrophoretic

investigations in the zero gravity environment of the space laboratory.

Our "inside" consultant is Sydney E. Salmon, MD, Professor and Head, Section of Hematology and Oncology, Department of Internal Medicine at the College of Medicine, University of Arizona. He will become involved during the clinical evaluation of the apparatus. He is well known for his investigations in clinical oncology.

Mr. L. Ralph Baker, Research Associate in Optical Sciences at the University of Arizona, is the senior electronics engineer on the program. He is expert in the design and assembly of the electronics and associated computer interfaces for the acquisition and display of image data utilizing television techniques under computer control. Mr. Baker was a senior research engineer on the Ranger and Mariner programs at the Jet Propulsion Laboratory, 1962-1967, and at the University of Arizona he designed, developed, and built the apparatus for the real-time display system for the Pioneer 10 and 11 encounters of the planet Jupiter that was used at NASA Ames Research Center in 1973 and 1974.

The Program Manager, Mr. Charles Blenman, Jr., has had extensive experience in engineering and in the administration of research in the Navy, in the aerospace industry, and at the Optical Sciences Center of the University of Arizona, where he has been employed for the past 10 years.

Mr. John Holcomb, a graduate student in Optical Sciences is the program's optomechanical engineer. He had done much original work in industry as a mechanical engineer, specifically in the area of microscope apparatus.

Mr. Kai-wah Chan, a graduate student in electrical engineering and computer sciences is the principal programmer for the development and test of the programs for the acquisition, display, and analysis of the electrophoresis

data. As of August 1977, his title was changed to Design Engineer, having completed his graduate studies leading to a Master's degree in electrical engineering.

We believe that we have an exceptionally well qualified team that has successfully undertaken the development of the automated analytical electrophoresis apparatus (AAEA), within the time and funds available to date.

D. Design Objectives and Technical Approach

During the first 12 months, our objectives have been to design and bread-board an automated analytical electrophoresis apparatus and to demonstrate the feasibility of the design. The apparatus should be capable of determining and displaying the spectrum of mobilities in a cell population suspended in a suitable buffer and contained within a microscope electrophoresis device. Over a longer term, our design objective is to provide a system for a sophisticated automated capability as a tool for both research and clinical use, and for support of the NASA Shuttle Electrophoresis Facility (NASEF) mission.

We proposed, assembled, and demonstrated an apparatus consisting of a microscope, with a computer-controlled focusing device, linked to a television camera and controlled by a computer. The computer scans and locks onto the phase-contrast image of a migrating cell and measures its migration velocity, i.e., displacement as a function of time. The data are to be digitally encoded and stored on disk for subsequent computer processing. The computer evaluates all data accumulated over a period of time and correlates all other known facts about the sample, such as the clinical diagnosis of the patient from whom the sample was obtained. The composition and operation of the present system are detailed in subsequent sections.

The AAEA is designed to provide and display essentially two types of data: histograms and verifications of effective cell separations. (a) Histograms, or the mobility distributions in the given sample, are of utmost importance for the utilization of preparative electrophoresis instrumentation. If one wishes to isolate a specific abnormal component of, for instance, a lymphocyte population in a pathological specimen, all fractions collected would have to be evaluated for the suspected abnormality, in the absence of prior information. To the contrary, if the histogram points beforehand to an abnormality in mobility distribution, the search can be focused on the subpopulation with abnormal mobility only, thereby realizing an enormous saving in time. (b) The best evidence for effectiveness of separations of cells obtained in the preparative electrophoresis apparatus. *It is this second use that is of particular importance for the NASA program.* If NASEF is ever realized, its operators will need to know as rapidly as possible if their preparative apparatus has indeed accomplished the purpose, i.e., if they have isolated the desired fraction. Manual microscope electrophoresis is completely unsuitable for such a purpose. Thus, it can be safely anticipated that the automated apparatus will be an indispensable part of NASEF. It should be emphasized, however, that AAEA will not require the shuttle facility for its operation and will be usable equally in the presence or absence of gravity.

There may be alternative ways to automate electrophoretic measurements such as the recently developed laser techniques. Without going into the limitations of the detectability of the minute changes in the light frequency caused by the Doppler effect at the low migration velocities of the cells in an electrical field, there is one argument that overwhelmingly slanted the

desired automation toward direct optical sensing. This is the ability to program the computer to recognize not only all the cells present, but also to differentiate cells according to artificially added *fluorescent markers*. The use of fluorescence-labelled antibody-markers has proven to be a most important tool in lymphocyte immunology, and the suggested technique would yield directly the mobility data on such labelled cells. The importance of this feature of the apparatus cannot be overemphasized. This capability will hopefully be added during a later stage of development.

During the first year of effort, the basic structure of the required software package was to be defined in a modular fashion. Specific program modules were implemented according to an order of priorities determined by the need of overall system development. For example, one of the first program modules was data acquisition, i.e., the reading of the vidicon and the clocking of migrating particles. The testing of data sets with specialized test statistics, on the other hand, was to be anticipated in the consideration of data formats and retrieval programs, but this was not attempted during the first year.

The programming followed the same design principles as employed in other computer-controlled microscopes, with user-transparency being the prime consideration. No special system commands are demanded of the user nor will they have to be learned by the user. Instead, frames in plain English will appear on a CRT and the user will merely select options from a hierarchy of programs. Fortran will be used exclusively for portability, ease of documentation, and modification by a user's personnel. Adequate documentation will be provided with the statement of purpose of each program section, lists of variables, logical flow charts, and source codes with comments.

Past experience has shown that ease of file handling is extremely important in biologic data manipulation. The operating system of the computer will be used for such activities, but again, with option selection frames and displayed instructions. Files will be formed, deleted, and merged. It will be possible to select from files by name of the datum or by property (i.e., all cells with a mobility in a certain range can be assembled into a subfile). The raw data acquisition of cell velocities will provide, in the first round of program development, for lock-on onto a single cell only, migrating within a narrow strip of the total field. Continuous velocity measurements will be recorded and stored; correction for angular motion in lateral directions within the narrow observation strip will be made, and a threshold will be observed for maintenance of contrast. This monitors the maintenance of migration at the prescribed channel depth. No velocity correction for angular movement in a vertical direction is planned at this time.

It will be necessary to establish confidence limits for entire mobility profiles. The profiles are considered as k -dimensional vectors, with k representing the number of histogram intervals. To accommodate the wide range of distributional variations expected with each interval for a given cell type, a multivariate beta distribution will be assumed. Programs to estimate the sample sizes required to establish confidence regions for such distributions, and to test for differences against other sets of distributions with controlled error of the first, as well as of the second kind, have been developed and will be adapted for use during the second and subsequent periods.

The design objective of demonstrating apparatus feasibility was attained in late May 1977 during a visit to the University of Arizona by the Technical Monitor, Dr. Robert Snyder.

We then commenced work on further developing the capability of the apparatus in respect to improved phase contrast imaging, elimination of vibration problems, design and installation of a water bath temperature control system, and further development of autofocus system electronics. We also continued to develop programs for system control operations and for data processing, utilizing a PDP 11/03 computer coupled to a PDP 11/45 computer. This was to prepare the system for integration to a dedicated Eclipse S/130 computer system that has been ordered and will be delivered in October 1977.

II. WORK ACCOMPLISHED DURING THE PERIOD

A. General

At contract go-ahead we commenced executing Task 1, which required designing all system components and interfaces, followed by submission of a drawing package and a list of equipment. This was completed in part by June 10, 1976, when the conceptual design was presented to the MFSC Technical Monitor along with definition of the major assemblies and subassemblies. Omitted, however, at that time were the designs of the autofocus unit and the optics of the microscope system. We had not then finished our literature search for likely autofocus techniques, and as the optics were straightforward, we deferred these designs until they were presented on September 2, 1976. In the meantime, we received approval on June 18th to procure the equipment prepared on June 10th. Approval for the equipment listed for the autofocus device and the optical system on September 2nd was received on September 13th. This completed Task 1.

Task 2 required the fabrication and test of a breadboard model of the system to demonstrate its feasibility. This task commenced on June 18, 1976, and was finally completed just before the visit of the Technical Monitor, Dr. Robert Snyder, to the University of Arizona on May 24, 1977. However, first assembly of the major equipments was done on April 12, 1977, and was shown to Dr. Snyder and to Dr. G. V. F. Seaman, our consultant. At this visit the optomechanical system was assembled and interfaced with the PDP 11/03 computer. Imagery of microscopic salt crystals on the interior surface of the electrophoresis chamber was displayed on the television monitors. The

autofocus system was not then operational, nor were the water bath's circulation and temperature control systems.

Tasks 2 and 3 were considered satisfied at the feasibility demonstration to Dr. Snyder on May 24, 1976, although vibration problems prevented satisfactory autofocus operation, and the displayed imagery was not optimum. Also the water circulation and temperature control systems, although on hand, had not been integrated into the whole AAEA. Moreover, there were hardware and software problems with the PDP 11/03 and 11/45 computers that had to be corrected by factory representatives.

The summer of 1977 through August 31st was devoted to correction of discrepancies existing at the feasibility demonstration that included improvement of phase contrast imagery, completion of the water bath circulation and temperature control system, elimination of vibration problems, work on autofocus unit problems, and completion and debugging of the computer operating system.

By late August we received procurement authority for the system's dedicated Data General S/130 computer and various peripherals. At this writing, delivery is expected by mid October.

Modification 4 to the contract dated 4 June 1977 provided incremental funding in the amount of \$25,000 through 31 August 1977 and provided the following tasks for the second period of performance, which ends on February 15, 1978.

"Background

Current space research activity in electrophoresis is directed toward the development of a NASA Shuttle Electrophoresis Facility for preparative

separation of living cells. Compared to the widespread use of electrophoresis in protein separations, electrophoresis of cells is still in its infancy. This is largely due to the limitations of presently available instruments suitable for cell electrophoresis."

"Statement of Work

The contractor is to design, develop, and build an automated analytical electrophoresis system that shall have the capabilities detailed in Appendix A of the Statement of Work.

The scope for this second year of effort follows the successful completion of the first year's tasks. The contractor shall interface a computer to the apparatus, and will then commence operational testing of the same to identify and correct problems that would impede meeting the performance specifications for the automated analytical electrophoresis apparatus.

Task 1. The contractor shall adapt and transfer the existing data acquisition software for the PDP 11/03 computer to a dedicated computer and transfer the existing "host" program for analytical PDP 11/45 subprograms to the computer.

Task 2. The contractor shall commence measurements of cell populations, monitoring the various performance parameters of the apparatus; correct deficiencies noted, including modifying the apparatus as required. Proposed major apparatus modifications shall be submitted to MSFC for approval.

Task 3. The contractor shall design, develop, and test analytical and data handling software."

(Modifications 4 and 5 to Contract NAS 8-31948 covering the second period of performance, including the above statement of work, were based on

proposal OSC 77-089 of June 3, 1977, which was in response to NASA MSFC RFQ No. 8-1-7-ES-07700-AP29C.)

On 23 August 1977, living red blood cells were introduced into the electrophoresis chamber and were satisfactorily displayed, as will be shown in subsequent sections.

In late August, Modification 5 to the contract was received, which added \$60,000 incrementally for the period 1 September to 30 November 1977. This, along with the \$25,000 received under Modification 4, raised the current contract value from \$83,780 to \$165,000. This \$60,000 increment permitted acquisition of the dedicated computer system, the integration of which will be a major endeavor during the second performance period.

In the subsequent sections, details are presented of the work accomplished to date in the areas of electronics and data processing and control hardware, software, and optomechanics.

B. Electronics and Data Processing and Control Hardware

Our first task in late May 1976 was to design the video compressor-computer interface. We started an analysis on the focus problem for the AAFA to allow us to make an intelligent decision on whether to make or buy. We contacted Colorado Video, Inc., the vendor for the video compressor, and were informed that the unit would be donated to the University at no cost.

During July 1976, as a result of our investigation into autofocusing techniques, we decided to build our own since no commercially available units met our budget or our requirements. We made another decision to use Dr. Bartels' PDP 11/03 computer, which had recently been purchased with funds from one of Dr. Bartels existing grants. We knew the 11/03 was too slow to

perform all the required tasks, but would work well enough to show feasibility until a dedicated computer could be purchased in the second performance period.

As we were analyzing the preliminary design for the video compressor interface, some deficiencies were uncovered, so we redesigned the interface making it more intelligent. This in turn, allowed more efficient programming.

The redesign of the video compressor interface and also the preliminary design of the autofocus unit were completed in August 1977. We started fabricating the interface, and some electronic parts were ordered.

In September 1976 a package of drawings was submitted to the Technical Monitor for design approval of the autofocus and redesigned interface units. We received some of the special equipment and continued with the fabrication of the interface.

The video compressor interface was completed next after some minor modifications were made to the digital-to-analog converter section. We were forced to make a design change in the voltage-controlled oscillator (VCO) section of the autofocus unit. The voltage-to-frequency converter integrated circuit we had planned to use would not then be available for several months, so we redesigned the VCO using discrete components. We received the stepper motor and stepper motor driver, which completed all the capital equipment deliveries. After a series of problems with the PDP 11/03, it became operational.

In order to better implement the AAEA system integration, we moved the AAEA including the computer in November 1976 to the electronics shop in the Optical Sciences Center.

We found a problem in obtaining the correct resistance and power level (~100 watts) of the necessary dropping resistors for the stepper motor. The

motor is an eight-phase motor that requires nine dropping resistors. We had not anticipated that these resistors would not be available locally, and we had to special order them after exhausting all sources of supply in the State of Arizona.

In December 1977 we discovered some minor cabling problems between the interface and the computer. Testing the interface proceeded slowly, but steadily. We continued to experience problems in locating the power resistors for the stepper motor as the manufacturer had discontinued them.

The video compressor was successfully operated in January 1977 by means of our interface and the 11/03 computer. Further minor modifications were made to the interface to bring the system up to operational status.

We gave a demonstration of the video part of the system to Drs. G. V. F. Seaman and Robert Knox, program consultants from the University of Oregon, in February 1977. A program was written to display scanned data from the TV camera on an LA30 Decwriter. This display was crude, as it was intended only to demonstrate that the video was being properly digitized and stored in the correct locations in the 11/03 memory.

We finally obtained the stepper motor power resistors in March 1977 through a colleague who was in the San Francisco area on other University business.

We also made some changes to the video processing section of the autofocus unit to improve the focus signal quality and in an attempt to improve autofocus operation.

Further changes were made to the autofocus electronics in April, but we discovered yet one more problem area in the integrator section. The integrator output had a random amplitude variation at a fairly low frequency

for which we could find no explanation. As mentioned previously, displays of microscope imagery were made on April 12th to Drs. Seaman and Snyder.

We returned the AAEA to Dr. Bartels' laboratory in the Microbiology Department. In his laboratory we found an excessive sensitivity to vibration due to the high magnification of the optical system. This vibration upset the autofocus system by adding systemic noise that resulted in false information being fed to the electronics.

We also had problems in data transmission between the PDP 11/45 and the 11/03. This problem considerably slowed program development at that time and required Digital Equipment Corporation factory assistance. On May 24, 1977 the system was sufficiently operational to indicate its feasibility to the Technical Monitor.

In June 1977 we commenced correcting systems deficiencies. We redesigned the integrator section of the autofocus electronics. The previous design integrated the focus signal for one TV field. The new design integrated for 10 TV fields, which dramatically improved autofocus operation. The noise in the integrator, discussed above, resulted from the noise in the video from the TV camera. Integrating over 10 TV fields reduced the noise in the integrator output to an insignificant level. The autofocus board was modified and checked out on the bench.

We tested the temperature controller for the water bath. It functioned very well.

In order that the AAEA computer should know the electrophoresis chamber temperature, we designed, fabricated, and tested a remote reading thermometer in July 1977. The thermometer has a small probe that will be placed in close proximity to the chamber.

We next made modifications to the electrophoresis chamber power supply design. The design now includes provision for operating chamber voltage (or current) magnitude and polarity manually. Thus, the computer need not be running to set the chamber electric field.

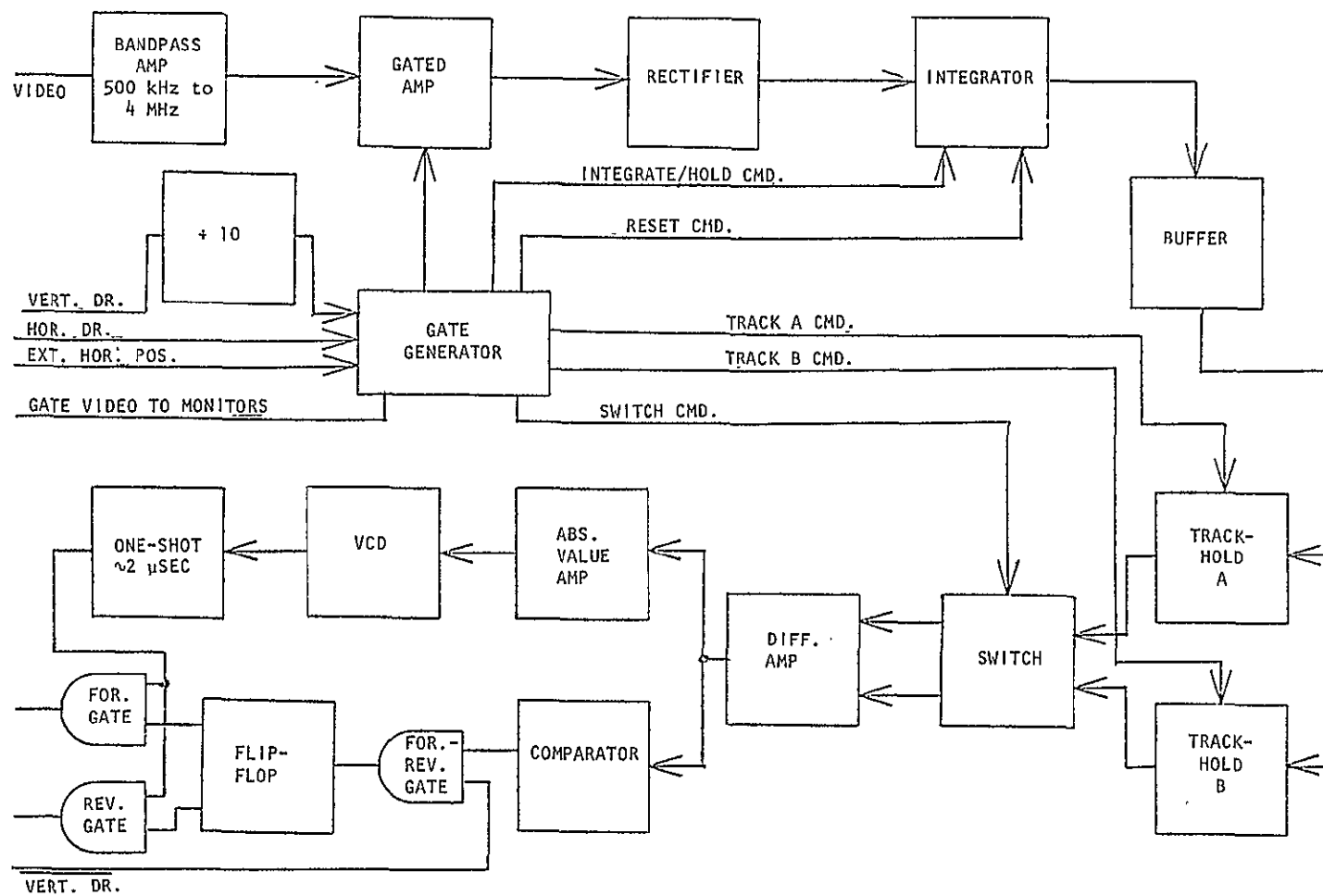
The redesigned autofocus electronics were checked out using a blood slide in late August and performed better.

We next placed the electrophoresis chamber in the water bath and viewed live red blood cells while operating the electrode power supply. Contrast was good, and the cells had some mobility. However, we were not looking at the correct depth in the electrophoresis chamber, and no automated tracking was attempted.

Description of Autofocus Unit

The autofocus unit (AFU) consists of two plug-in circuit cards designed to process the video signal to generate a forward step pulse (FOR) or a reverse step pulse (REV) to the stepper motor driver. The stepper motor drives the microscope fine focus stage to keep the image of the cells in focus at all times. Figure 1 is a block diagram of the basic autofocus configuration.

Referring to Fig. 2, the video from the TV camera is fed to IC1, which is a high-pass filter with a lower cutoff frequency of 500 kHz. The output from IC1 then passes through gated amplifier IC-26, whose gate signal is described later. IC26 is gated to remove all sync and video signals from areas in the TV raster that are not used for focus determination, so only the video in the region of interest is amplified. The gated video signal then passes to IC2, which is a gain stage that forms a low impedance source for a halfwave rectifier. The output of the rectifier now contains unipolar



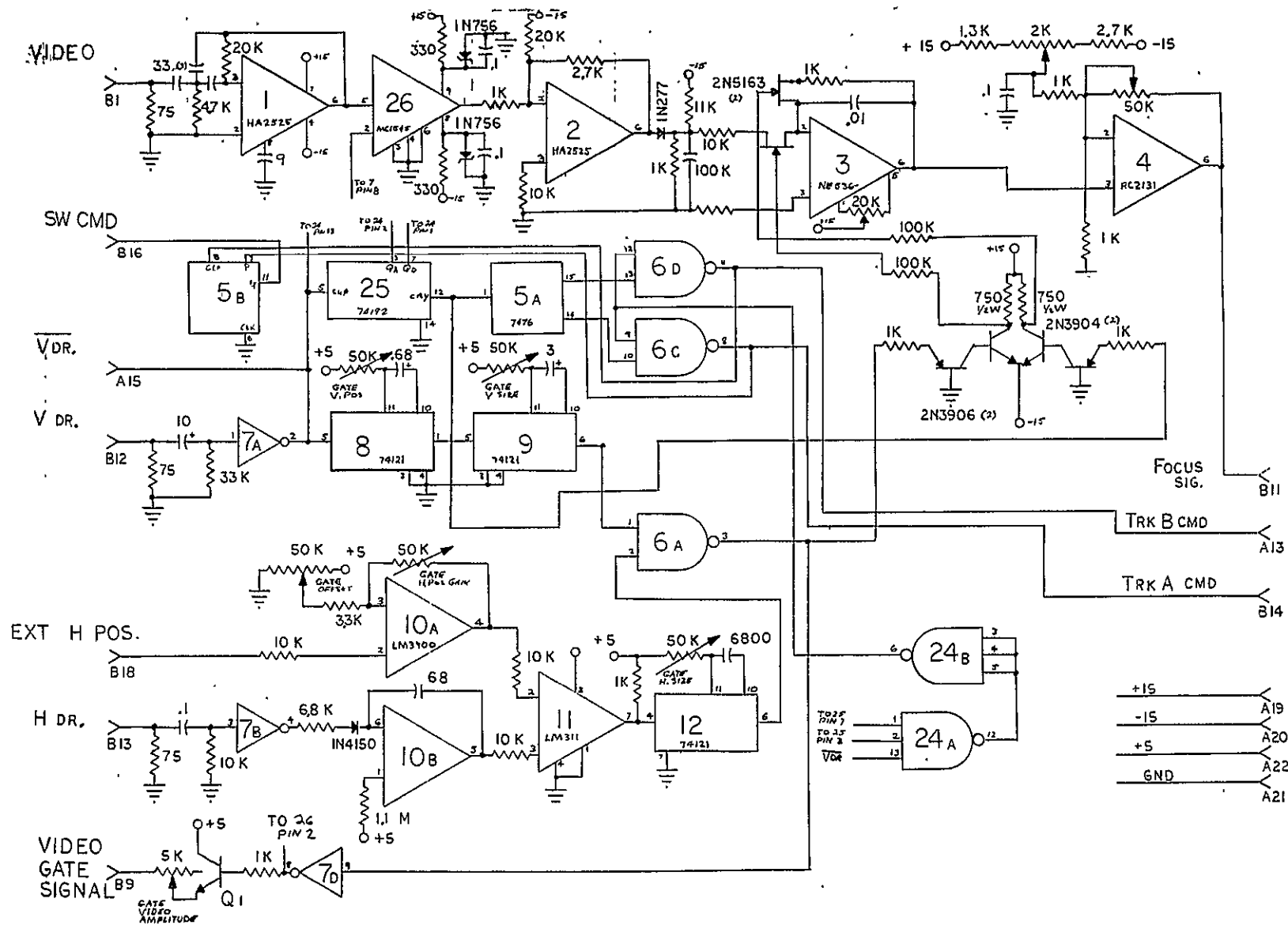
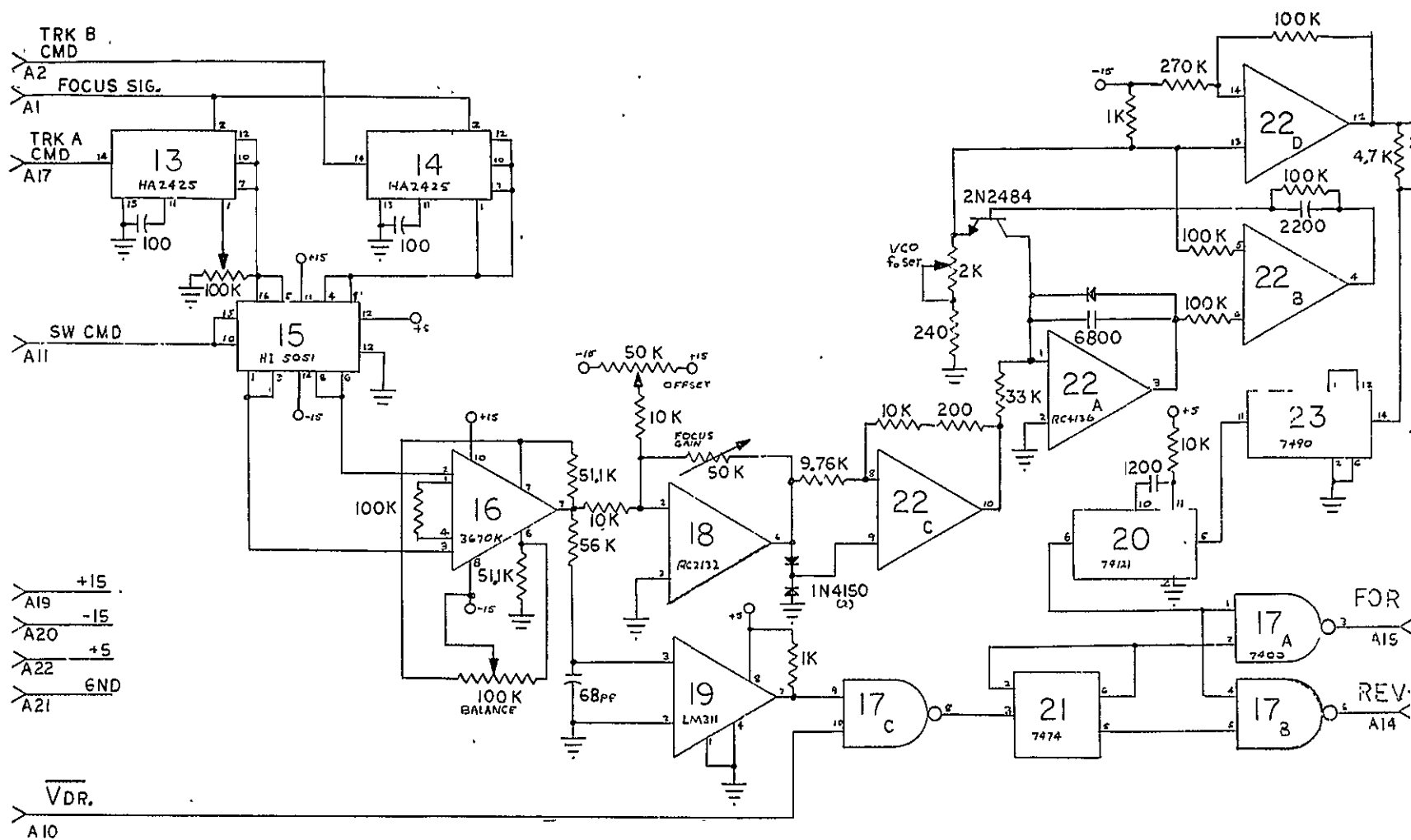


Fig. 2. AAEA autofocus unit, card 1.

high-frequency information that is integrated by IC3. The two FET switches control the integrator operation with three distinct modes of operation: integrate, hold, and reset. The time during which integration takes place is determined by the logic command that is derived by IC's 7A, 8, 9, 6A, 7B, 10A, 10B, 11, 12, and 25. IC4 is a buffer amplifier with a variable dc offset and gain that feeds the integrated FOCUS SIGNAL to card 2 (Fig. 3).

The gate signal is formed by taking vertical drive, V DR, from the EIA sync generator, level shifting and inverting it to trigger IC8. IC8 is a one-shot whose time is trimmer adjustable, which varies the vertical position of the gate within the TV raster. The gate vertical size is controlled by another one-shot, IC9. The horizontal portion of the gate signal is formed by taking horizontal drive, H DR, level shifting and inverting, then triggering IC10B, a ramp generator. The timing of the ramp is the duration of one horizontal TV line or 63.5 μ sec. The ramp and a variable dc level, EXT H POS, from the video compressor are compared in IC11. Before comparing, EXT H POS is buffered in IC10A to provide dc offset and gain for matching the cursor position from the video compressor in such a manner that the gate signal will track along with the cursor under program control.

When the two inputs to IC11 are equal, IC11 changes state, which triggers one-shot IC12. The time of IC12 is adjustable to vary the horizontal size of the gate signal. The output from IC12 and IC9 are "nanded" to form the composite gate signal which is then routed to the integrator (via the transistor switch), IC7D, and the gated amplifier, IC26. The output of IC7D is fed to a current amplifier, Q₁, which drives the gate signal on the video feed to the TV monitors to allow the operator to see where the computer is positioning the cursor and integration (gate) window.



The output from IC7A, \overline{VDR} , is fed to IC25, a divide-by-10 counter, which puts out a single pulse every 10 field pulses. This pulse is used to reset the integrator, and also toggles flip-flop IC5A. The toggling action of IC5A alternately turns on gates 6C and 6D. The output of 6C is a pulse occurring just before the reset pulse and is used to command track/hold A, IC13, on card 2. Likewise the output of 6D is a pulse occurring just before the reset pulse after an additional 10 field pulses, and forms the track command for track/hold B, IC14, on card 2. In this manner the focus signal is held for 20 fields in each track/hold unit, but is changed, alternately, every 10 fields.

IC5B is an R-S flip-flop that is set by TRK A CMD and reset by TRK B CMD. The output from 5B is therefore a square wave of 20 field period and is fed to IC15 on card 2. IC15 is an electronic switch wired in a way to cross-couple the output from 13 and 14 to the differential amplifier, IC16. The difference signal from IC16 goes to IC18, which is a variable offset, variable gain buffer amplifier. IC22 is an absolute value amplifier, which amplifies the *magnitude* of the difference of A and B, not the sign. The sign of the difference is determined by comparator IC19. IC's 22A, B, and C form a voltage-controlled oscillator that is used to control stepper motor speed. IC23 divides the VCO frequency by 10, and IC20 is a one-shot pulse standardizer for feeding standard width pulses to the stepper motor driver. IC21 is a flip-flop that controls the direction the motor drives by enabling either gate 17A or 17B.

Autofocus Operation. When high frequency video information is present in the window area, it is amplified, filtered, rectified, and integrated for 10 TV fields. Just before the integrator reset pulse occurs, a command is generated to cause track/hold (IC13) to track the focus signal for about

10 μ sec, then holds the signal until commanded to track again. Ten fields later, track/hold B is commanded to track for about 10 μ sec. The difference between the two held signals is amplified by IC16. IC22C amplifies the magnitude of the difference signal, not the sign of the signal. Thus, if the image is in focus, there will be no difference between A and B, and the motor will turn at a very slow speed, since the VCO frequency is proportional to the magnitude of the difference. If the image is moving off focus, the output from A or B will be less than before. If the motor is being driven in the wrong direction, A will be less than B, and the comparator will detect the change in sign and cause the motor direction to be reversed. The next sampling period is done with the inputs to IC16 reversed. Thus, if the motor is driving in the correct direction, $B > A$. If the reverse condition exists, the motor direction will be reversed. The inputs to the differential amplifier are always being reversed to maintain the conditions to cause the motor to always drive in the correct direction. As the magnitude of $(B-A)$ is sensed, the motor speed adjusts in a manner to slow down for small differences in focus, helping to maintain accurate focus and reducing vibration at the same time.

Description of Electrophoresis Chamber Power Supply

Referring to the AAEA electrophoresis chamber power supply schematic (Fig. 4), the power supply for the electrophoresis chamber is designed to be both computer and manually controlled.

The input from the computer comes from a 12-bit digital word for magnitude, and a single bit for polarity, making in effect a 13-bit D to A converter. The DAC output is a current (0 to -2 mA), which is connected to a

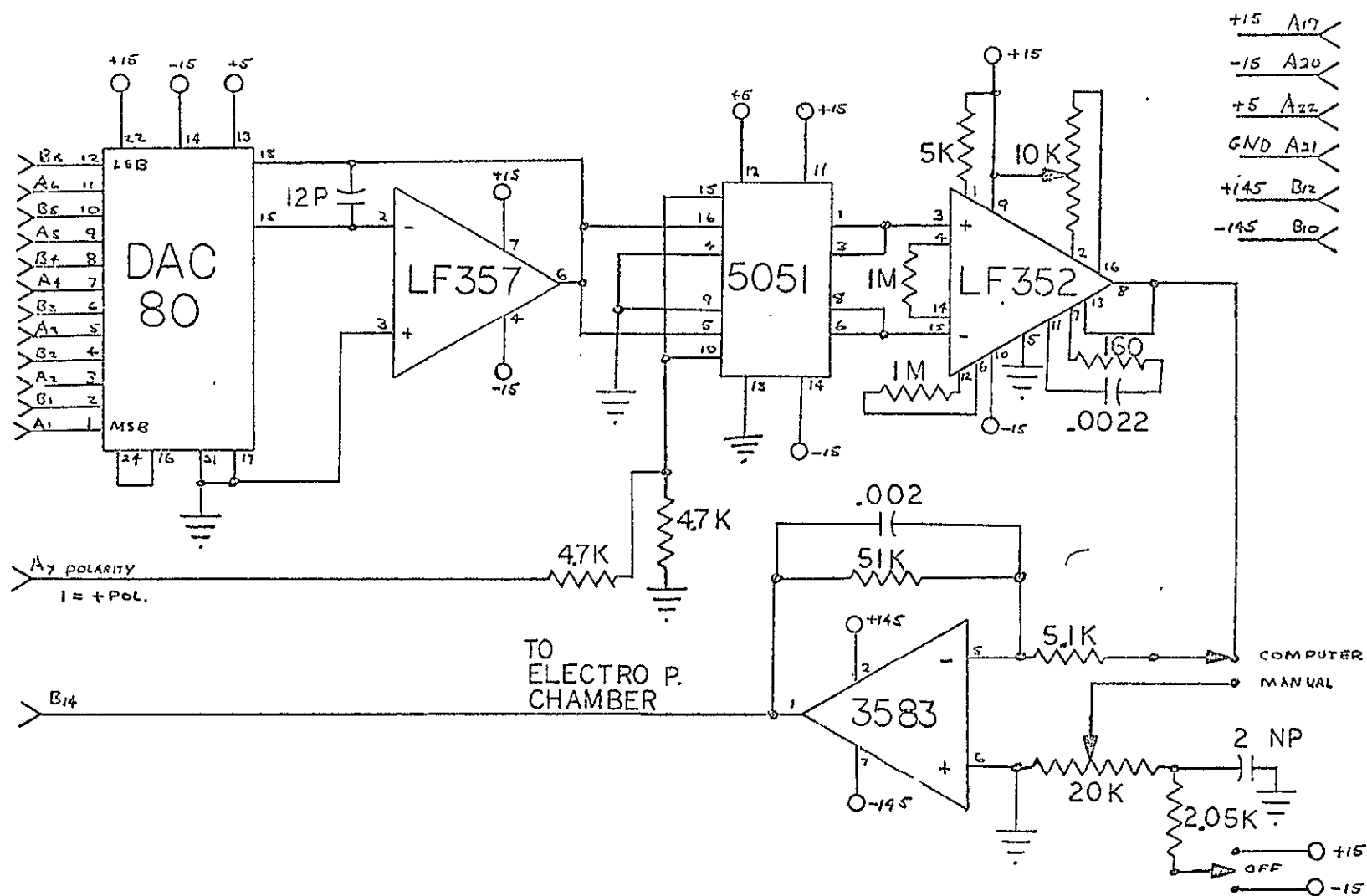


Fig. 4. AAEA electrophoresis chamber power supply.

current input amplifier (LF357). The output from the LF357 is a voltage from 0 to +10 V that is fed to an electronic switch (5051). The 5051 is connected as a dual DPST switch and is controlled by the polarity bit. The switch output is connected to both inputs of an LF352 instrumentation amplifier. When the polarity command is set to a "1," the output from the DAC is connected to the inverting input of the LF352, and the noninverting input is grounded, causing the output of the LF352 to be inverted, or 0 to -10 V. When the polarity command goes to a "0," the switch causes the DAC to be connected to the noninverting input and the inverting input to be grounded. Thus, the output from the LF352 is from 0 to +10 V.

The output from the LF352 goes to a toggle switch mounted on the rear of the card for ease of operation. In the computer mode, the 0 to ± 10 V signal feeds a high-voltage operational amplifier (3583) that is capable of ± 130 V, ± 75 mA output. The 3583 has a gain of 10, causing the chamber voltage to vary over the range of ± 100 V, depending on the state of input of the DAC and the polarity bit. In manual mode, the toggle switch is set on manual and a 10-turn pot adjusts the voltage. Another toggle switch sets the voltage to "+," "-", or "OFF." In manual mode, the chamber electric field can be adjusted without the computer running.

Two three-and-a-half-digit digital displays are mounted at the water bath tank to display chamber voltage *and* current so that the operator may observe these parameters at a glance.

Description of Water Bath Temperature Measurement and Control System

Referring to Fig. 5, the AAEA temperature measurement schematic, the thermometer consists of three operational amplifiers and two voltage regulators.

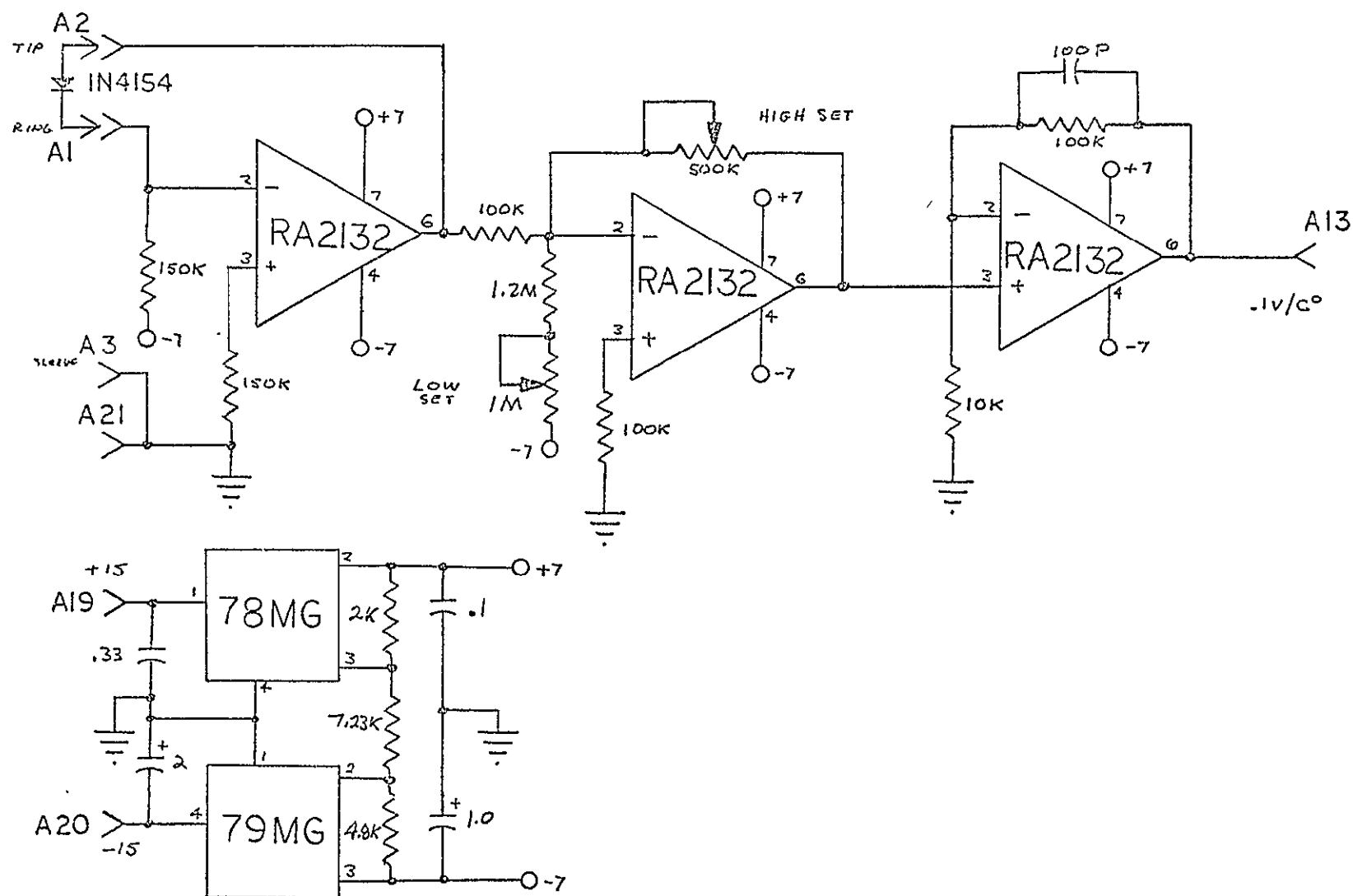


Fig. 5. AAEA temperature measurement.

The probe is a silicon diode that has very well known temperature characteristics.

The first operational amplifier acts like a simple constant current source for the diode probe. The noninverting input is grounded through a 150 k Ω resistor, so the operational amplifier output always moves sufficiently positive to keep the inverting input at ground potential as well. Thus, the current through the diode (via 150 k Ω to -7 V) is set to about 50 μ A by the -7 V supply and the 150 k resistor. Virtually all the constant current flows through the diode, and the voltage drop across the diode then depends only on temperature. Hence, the output of the first operational amplifier is proportional to the absolute temperature.

In order to correct the temperature range to C^o, the second operational amplifier is used to offset the diode voltage to give correct readings in degrees Celsius. The "low set" pot sets the output of the second operational amplifier to 0 V at 0^oC. The "high set" pot sets the operational amplifier gain and thus the correct voltage for the desired temperature range, 0.01 V per degree Celsius. The third operational amplifier is a gain stage of 10 to scale the reading to 0.1 V/^oC. The computer analog-to-digital converter has 12-bit accuracy and 5 V input, allowing temperature measurements of better than 0.01^oC.

The two regulators are simply to regulate the ± 15 V to ± 7 V for a very stable voltage source for the thermometer.

Description of Sync Generator

The AAEA sync generator (Fig. 6) is used to form the necessary synchronizing pulses to operate the TV camera, video processor, video compressor, and TV and waveform monitors.

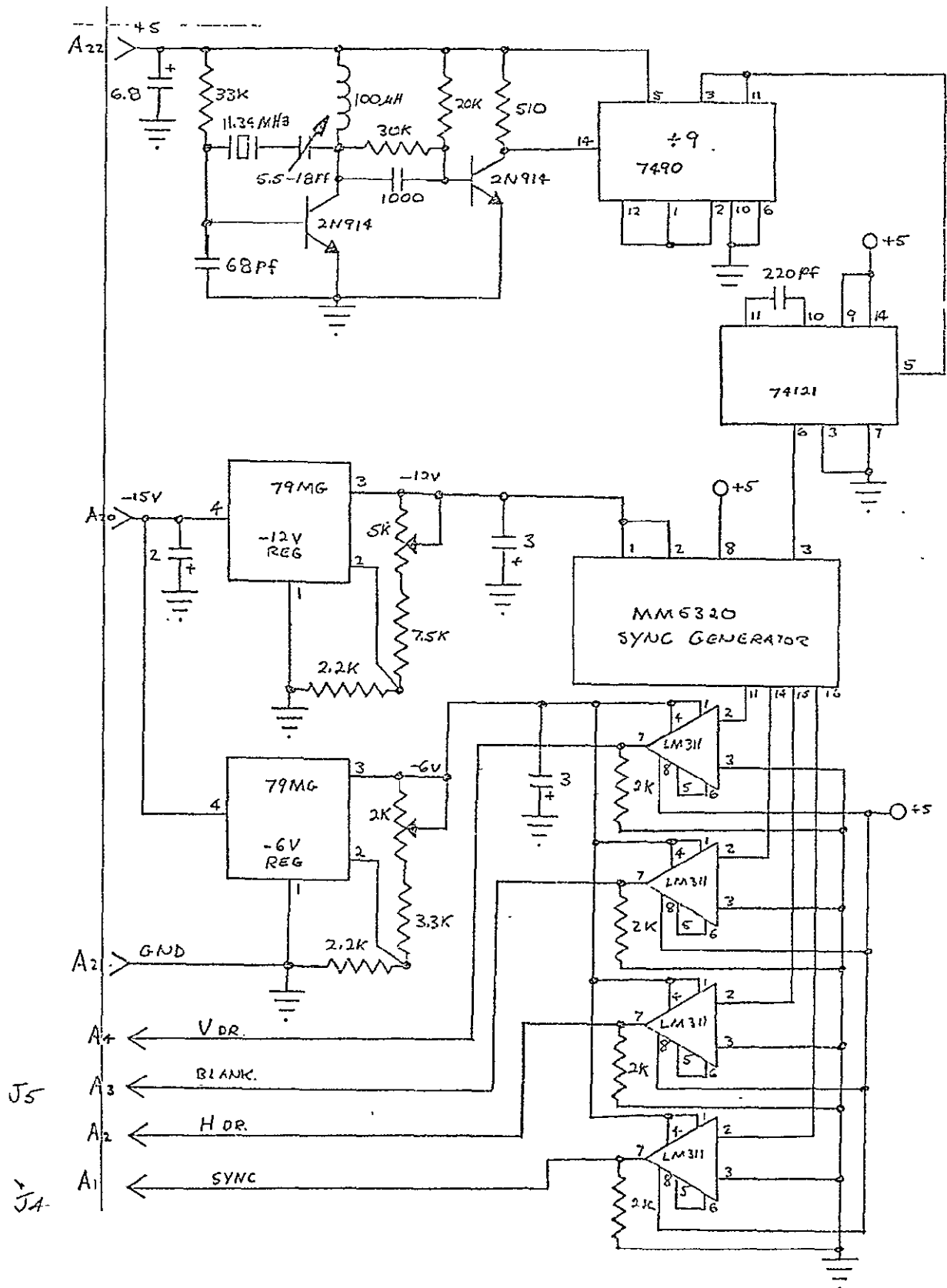


Fig. 6. AAEA sync generator.

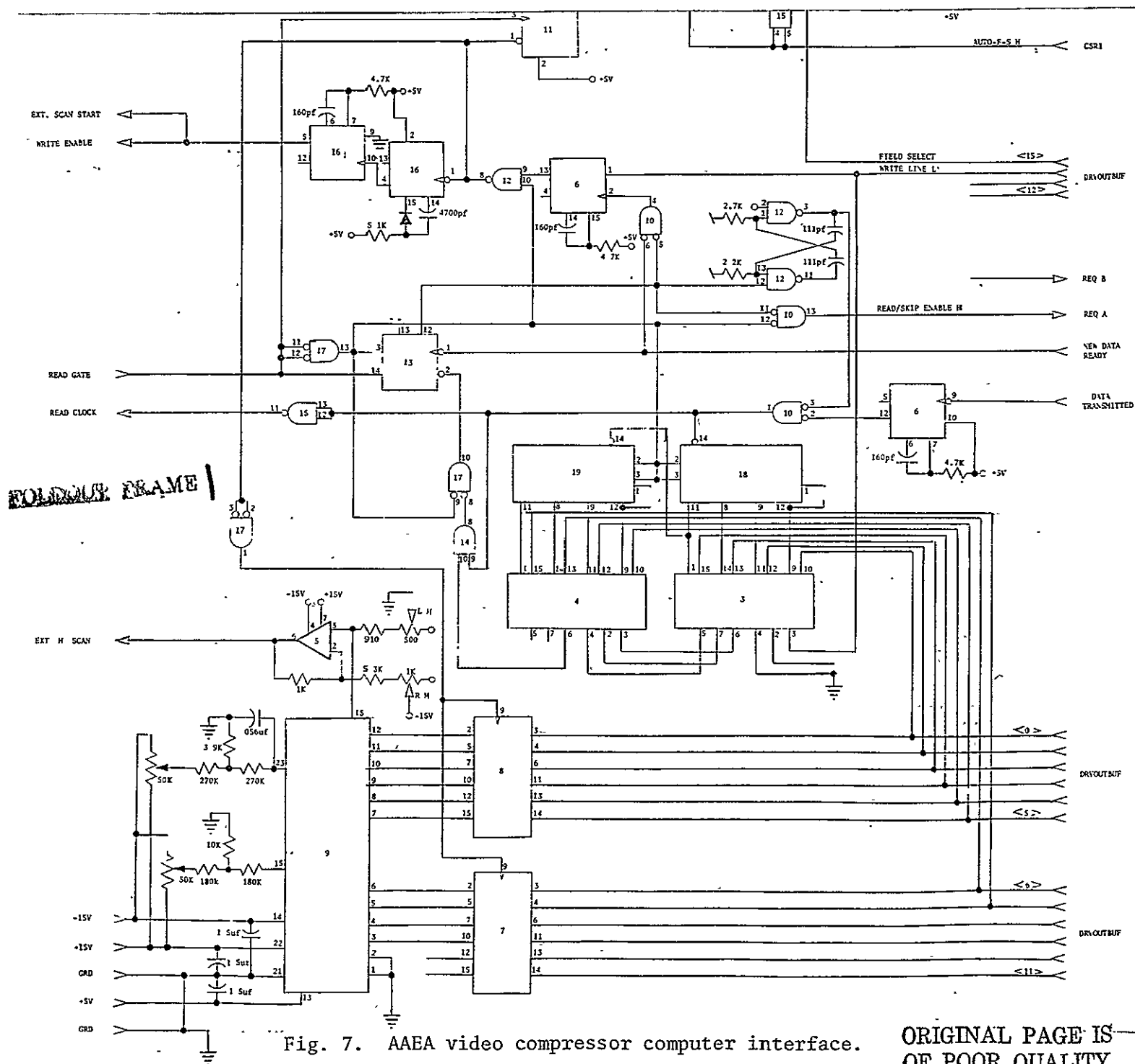
The two transistors form a crystal-controlled stable oscillator for the basic sync clock. The clock is divided by a factor of 9 to result in the correct frequency of 1.260 MHz. The divide-by-9 feeds a one-shot to square up the duty cycle of the basic clock frequency to about 50%. The sync, blanking, and drive signals are all generated in the sync generator chip MM5320. Standard EIA sync levels (0 to -4 V) are formed using LM311 comparators. The 311 has a very high drive current capability and is an excellent cable driver. The two regulators take -15 V input and form -12 V for the sync generator chip and -6 V for the EIA sync drivers.

Description of Video Compressor Interface

The video compressor computer interface (VCCI) is designed to video-to-computer interface compatibility, supplementary control functions, and more efficient data acquisition (see Fig. 7). The VCCI is designed around a random logic circuit rather than a clock-mode circuit. Random logic is used because the input-output signals are not in clock-mode, and this unit is required to have fast speed with a minimum number of chips. Since the VCCI is not a clock-mode circuit, the hazards of non-clock-mode logic were taken into consideration in the design. One major feature of the VCCI design is the division of the 16-bit output data lines from the DRV11C into two fields: command field and data field. Since there are only computer-controlled command lines, CSR1 and CSR2 can be used as command-control bits. They are not sufficient to request various operational functions. Splitting the 16-bit data into four command bits and 12 data bits provides additional functional controls. Moreover, it speeds up the operation by reducing two or more instructions into a single instruction.

The VCCI has six functional blocks: interface adapter, read/write control logic, buffer counter, external H (EXT H) scan generator, field index selector, and compressor timer. The interface adapter is simply a monostable multivibrator, IC6, that transforms a trailing edge signal from the computer digital output module, DRV11, into a 300 ns positive pulse.

The read/write control logic can issue read, skip, or write function commands. It consists of IC 10, 12, 13, and 16. A pair of NAND gates on IC12 form a 13 MHz oscillator. A flip-flop on IC13 and two other logic gates form the read and skip control section. A pair of monostable multivibrators, IC16, and other related logic gates form a "write-after-buffer-empty" control unit. Output line of IC10-pin 13 is a READ/SKIP ZENABLE H line telling the computer that video data are ready to be read or skipped when this line is high. When a read, MOV, video data instruction is issued, a pulse transformed from the DATA TRANSMITTED line triggers the buffer counter and is transmitted to the video compressor. A skip command is a "high" level sent on DRVOUTBUF<14> (WRITE LINE L). The trailing edge of NEW DATA READY triggers a skip operation if the value of the buffer counter is less than that of the lower byte of the DRVOUTBUF; otherwise, an empty buffer operation is performed. If a "low" level signal is sent on the WRITE LINE L line, a pulse is triggered by the NEW DATA READY command on IC6-pin 13. The pulse is blocked by IC12/8 if the data buffer is not empty. The pulse transmission is continued after an automatic buffer clear is finished. The pulse is then delayed by IC16 for about 5 μ s to allow the EXT H scan to be ready. Lastly, the pulse appears on the WRITE ENABLE and EXTERNAL SCAN (EXT) START lines of the video compressor.



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The buffer counter is composed of two binary counters, IC18 and IC19, and two magnitude comparators, IC3 and IC4. This unit keeps track of the number of video elements being accepted and also allows skipping video data to a certain position specified by the lower byte of DRVOUTBUF.

EXT H scan generator is on the lower left corner of the diagram. It is constructed by two 6-bit latch registers, a digital-to-analog converter (DAC) and an operational amplifier. The digital cursor position is stored into the latch register in a WRITE operation and the DAC converter and amplifier converts the digital word into an analog signal that is connected to the EXT H scan line.

Field-index selector is composed of a pair of flip-flops on IC11 and logic gates on IC15. Video scan field can be selected either automatically or selectively. When the automatic selection is disabled, DRVOUTBUF<15> specifies which field is desired. In automatic operation, the next field is selected.

IC1 and IC2 form the compressor timer that provides a maximum 8.53 sec timing interval with an increment of 1/30 sec. It is driven by the vertical frame sync and reset by CSRO (TIMER RESET H line).

C. Software

During the first year of effort, the basic structure of the required software package was written in a modular fashion. Specific program modules were implemented according to an order of priorities determined by the need of overall system development. For example, one of the first program modules was video data acquisition. This program printed out on the teleprinter a crude picture of a vidicon-scanned image to demonstrate the data

acquisition capability. Since application program development could not proceed on a microcomputer without an operating system, the development of such a system was a major and high-priority module in our first year of programming.

Software programming can be categorized into four major groups: system macros, system subroutines, operating system program, and testing/demonstration programs. All programs were written on the minicomputer PDP 11/45 and then were transferred and debugged on the microcomputer PDP 11/03. The purpose of the programming was to provide necessary routines for the blind microcomputer for communication with the PDP 11/03 and for system operation.

The macro package has the following program names: CICW, DEF, DLVIN, DLVOUT, ACCEPT, TYPE, TYPES, PRINT, EXIT, MBIOCT, OCTMBI, COM45, MTPS, EXIT, SETC, ZMPTC, and STRC. A major portion of the package is for terminal I/O facilities. The macros whose last letter is "C" are video scan command macros. The macros are usually equipped with conditional macro sections such that parameters can be entered to specify various desired functional expressions.

The subroutine package contains program subroutines and interrupt service subroutines. The program subroutines are TRANS, TYPE, MBIOCT, OCTMBI, COM45, LOAD03, and DMP45. The interrupt service subroutines are CONN, CONOUT, COMTER, ZRRMEM, ZRRINS, POWFL, and POWUF.

The operating system program coordinates the communication between the microcomputer, the user, and the PDP 11/45 computer system. The structure of this program is shown in Fig. 8. The program contains six major section commands: GET, RUN, DUMP, STATUS, DEBUG, and HELP. GET loads an object file from PDP 11/45 into PDP 11/03 memory core. RUN initiates the

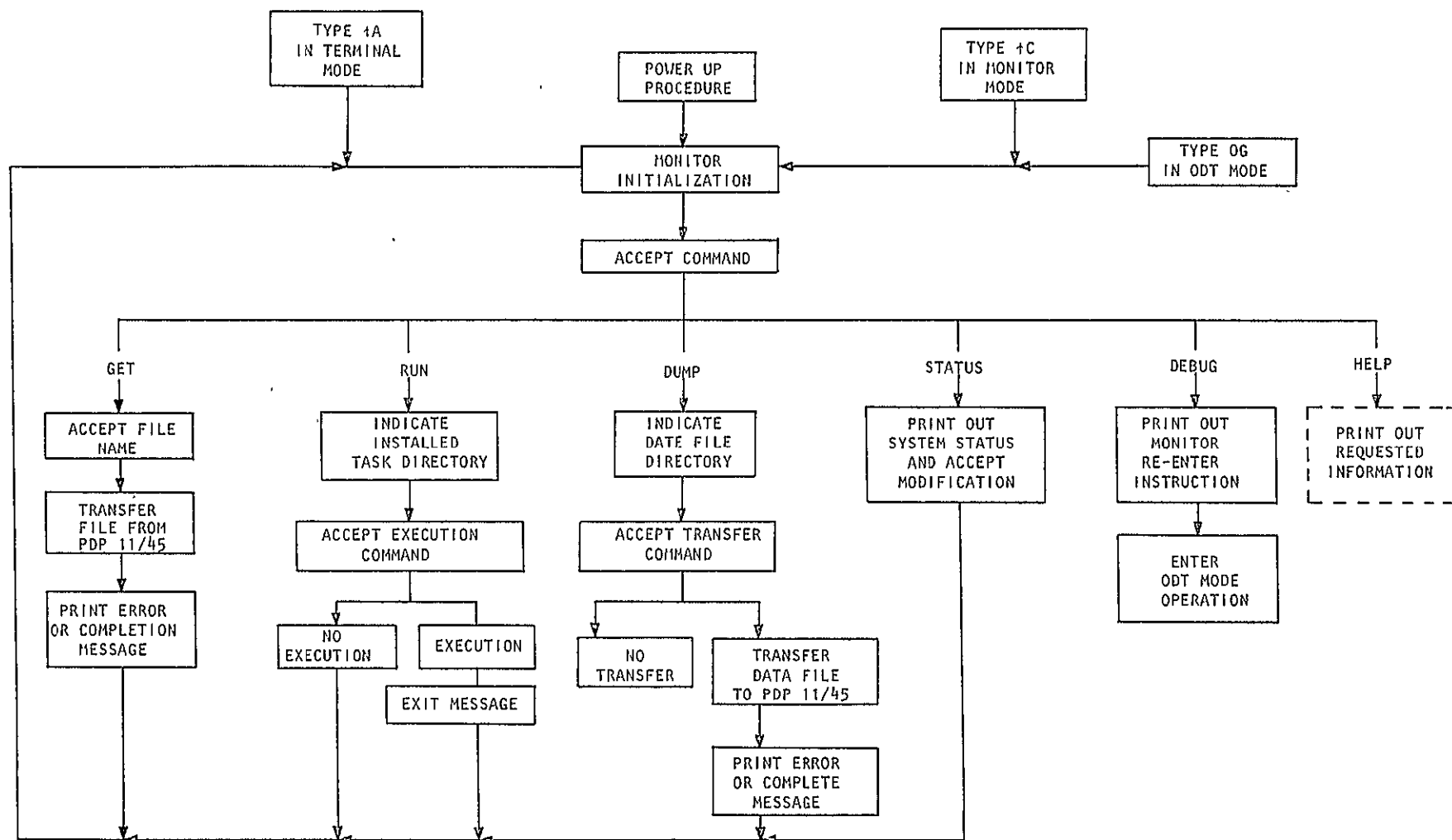


Fig. 8. PDP 11/03 operating system structure diagram.

execution of an installed task. DUMP transfers available data files to minicomputer disk device. STATUS indicates the current system status and allows changes. DEBUG allows the on-line debugging techniques (ODT) operations. Finally, HELP will guide a new user to run the system. The philosophy of the program design is to provide a sequence of "do-it-yourself" instructions to the user such that the complicated computer programming becomes transparent to the general operator.

A number of test programs for the software development and hardware installation checkout have been written, updated, and deleted. Some of the application programs are undergoing development, such as the cell recognition and tracking programs. By mid October 1977 we plan to have the cell recognition program finished and have the cell tracking program under development.

The programming follows the same design principles as used in other computer-controlled microscopes, with user-transparency being the prime consideration. No special system commands are demanded of the operator nor have to be learned by him. Instead, frames in plain English will appear on a console CRT and the operator will merely select options from a hierarchy of programs. Fortran IV is used extensively for portability, ease of documentation, and modification by operating personnel.

D. Optomechanical

The basic design of the microscope optics was essentially as proposed. Our original idea was to modify the Rank Brothers (England) electrophoresis microscope to our needs. One such apparatus was ordered in June 1976. In August Dr. Bartels redefined the design of the microscope optics and provided

a list of optical components, most of which were of Carl Zeiss, Inc., manufacture. In early September 1976 the optical system design along with its equipment list was forwarded to the Technical Monitor for his approval. This was received about 15 September, and equipment procurement was initiated. Delays in the delivery of the Zeiss components were experienced until late December when all were received.

In the interim it was decided that an optimum system would consist primarily of Carl Zeiss equipment as a phase contrast technique was required to best allow the autofocus system to function. Accordingly, parts of the Rank electrophoresis assembly consisting of the water tank, including the chamber support assembly, the lamp housing, and the electrophoresis chamber, and illuminator power supplies, were used along with the Carl Zeiss optics. Furthermore, as our design required very close water bath temperature control, which the Rank apparatus could not offer, we elected to design and fabricate our own water circulation and temperature control system, which would also operate with less vibration.

Work was initiated in January 1977 to combine the optical design and the optical and mechanical components of the AAEA into a functional unit. This design work continued into February. Work done in March and in the months following was in the nature of additions, changes, or modifications to the then existing system design. Most of the instrument shop work on the basic assembly was completed during the month of March. Thereafter shop work was done to make modifications and specific additions to the system. Enough of this work was completed by the second week in April to have the unit assembled for limited demonstrations to the Technical Monitor and to Dr. G. V. F. Seaman, our consultant.

The descriptions of the major optomechanical assemblies and their components follow.

Basic Design: Optical System Components

The basic optical design is shown in Fig. 9. Figures 10, 11, 12, and 13 are photographs of the assembled optical and mechanical components. Figures 14 and 15 are drawings of assemblies not shown in the photographs. In the following subsections, numbers in parentheses indicate specific locations on the photographs or figures.

Phase Contrast Objective and Condenser. In this instrument transmitted light phase contrast optics are used to provide high-contrast image detail for use with the video camera. The objective used for this system is the Zeiss 40X phase contrast objective Ph2/0.65 with small upper hemisphere. This objective must be used with a phase contrast condenser. The annular condenser stop is imaged directly onto the objective phase ring. For the condenser a single element lens is used with a centerable and focusable annular stop.

A special sealed housing for the objective and the hemisphere was made to prevent water from entering the space between these two components. This housing is made so that the objective may be used without its hemisphere when a lower magnification is needed. A sealed housing was also made for the condenser to prevent water from entering the airspace at the condenser's rear surface.

Tube Lens. The end of the objective housing opposite the objective was designed to hold a tube lens for correcting the image location in the elongated tube. The objective housing's dovetail ring fastens to the corresponding part on the epi-microscope body.

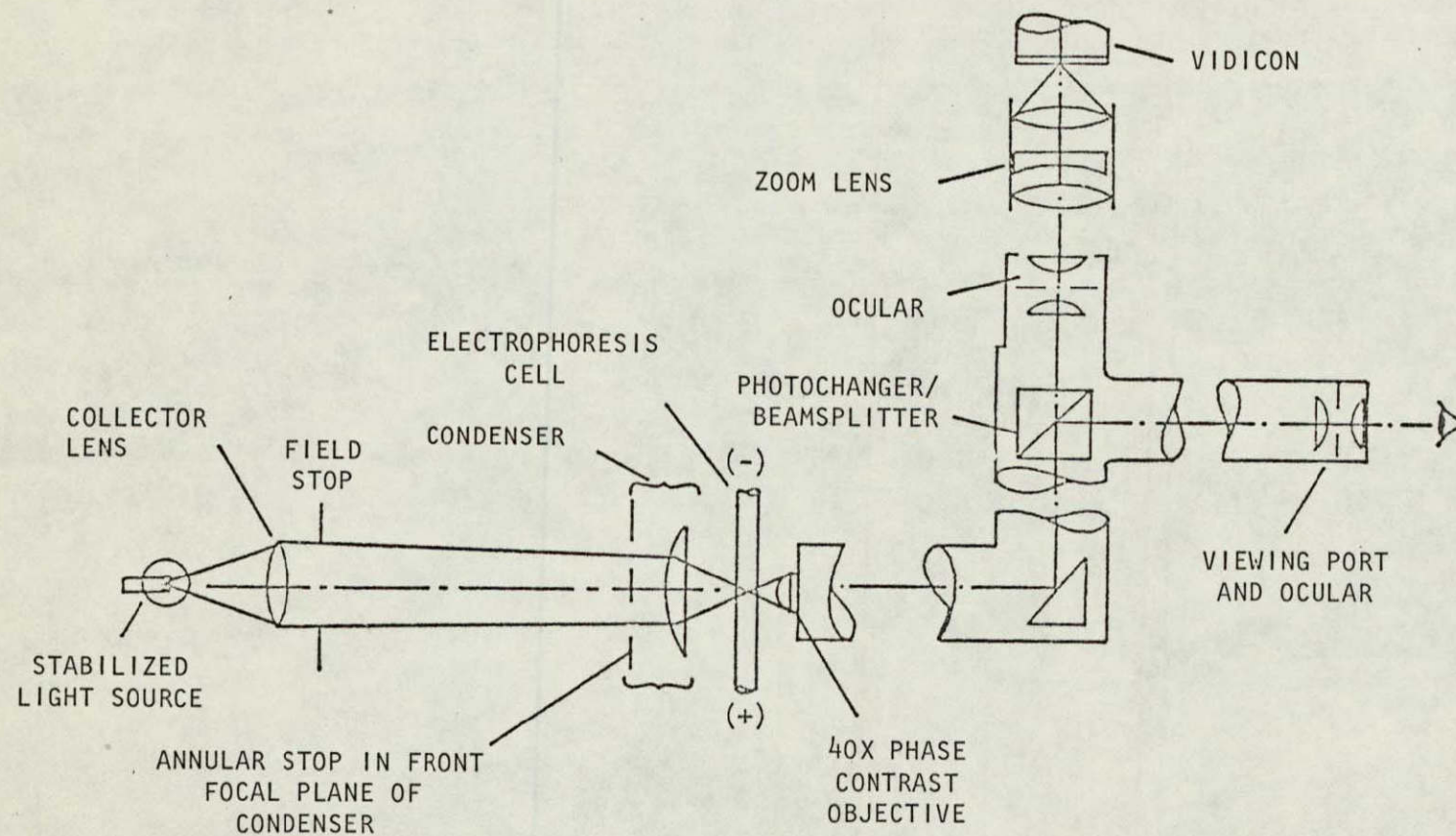


Fig. 9. Schematic diagram--optical system of automated analytical electrophoresis apparatus.

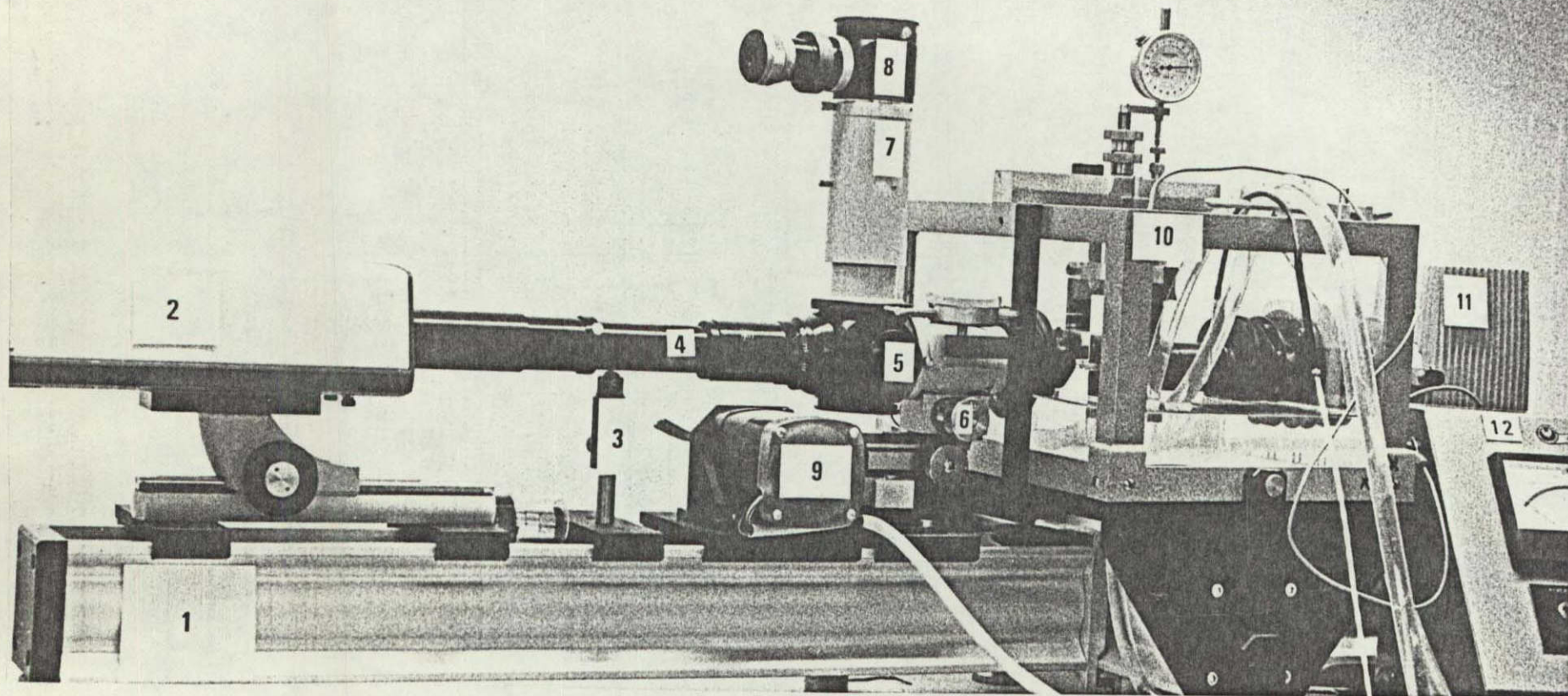


Fig. 10. AAEA optical bench, showing TV camera (2), microscope optics (4,5,6,7,8), stepper motor (9), "fish tank" (10), light source (11), and the Rank power supply (12). Note: in final configuration, the Rank power supply will be replaced by a computer-controlled power supply.

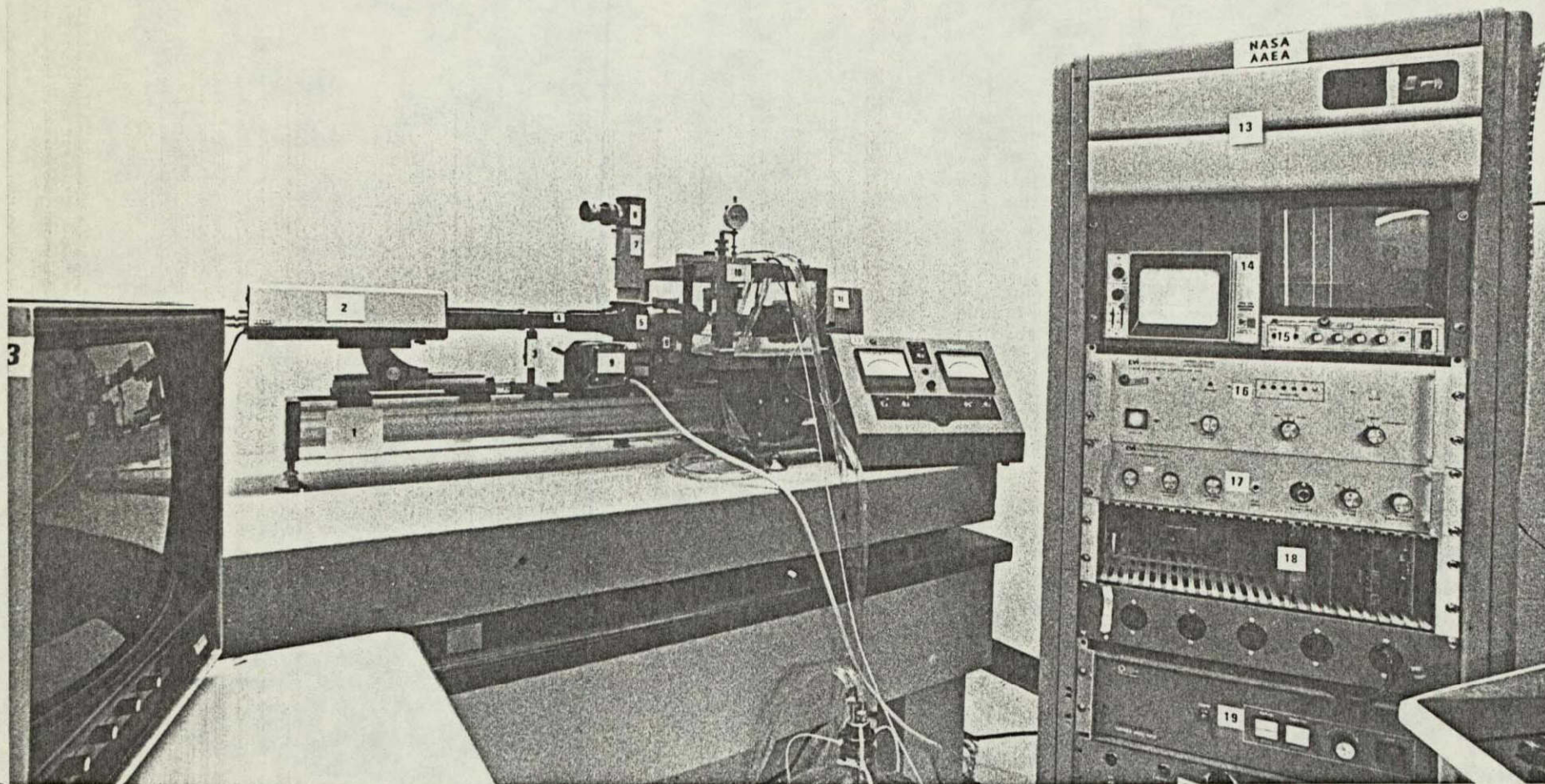


Fig. 11. AAEA system, showing the 17-in. TV monitor (23), and the electronics rack that contains the PDP 11/03 computer (13), waveform monitor (14), picture monitor (15), video compressor (16), video processor (17), electronics card cage (for AFU, interface, and sync generator) (18), stepper motor driver (19), temperature controller (20), and water ballast (22). (Not shown is the resistor bank (21) at bottom of console.)

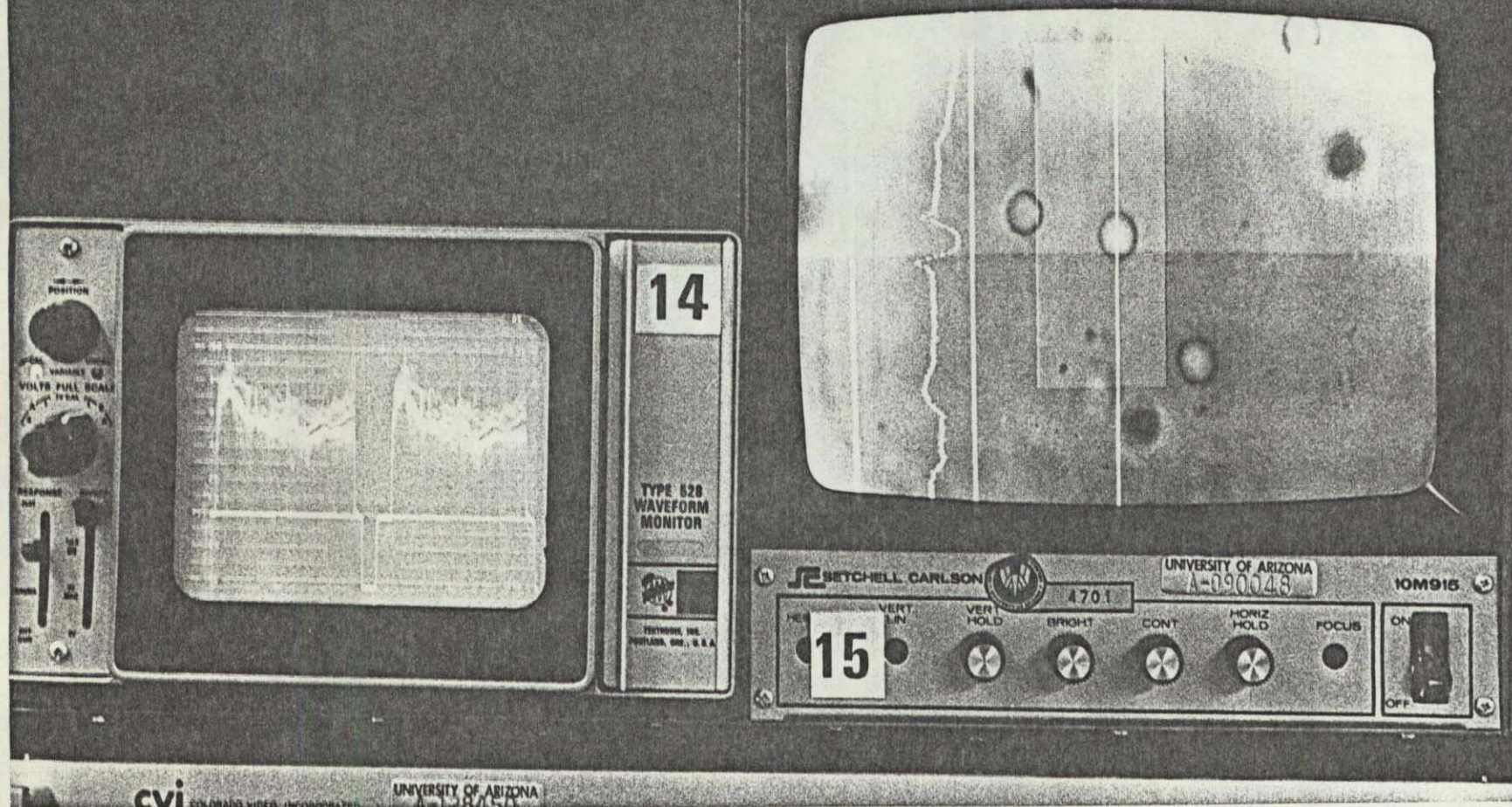


Fig. 12. AAEA video and waveform TV monitors. The picture monitor (15) shows a red blood cell under the cursor and the display of the video intensity along the cursor, on the left side of the raster. The waveform monitor (14) shows the video signal amplitude for the TV picture as shown.

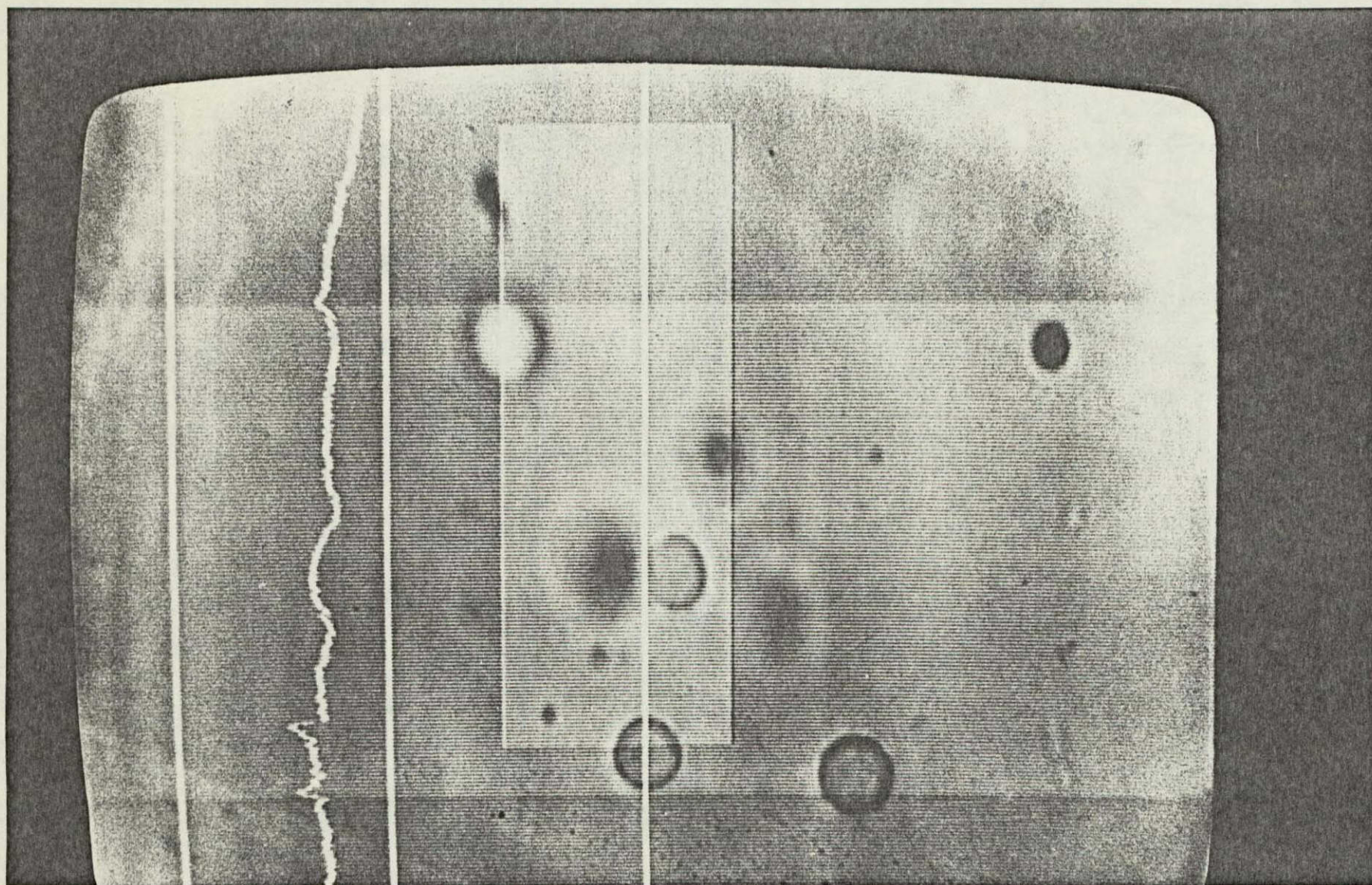


Fig. 13. AAEA closeup of the TV monitor showing red blood cells, the cursor, the video gate signal, and the display of video amplitude (wavy line) along the cursor.

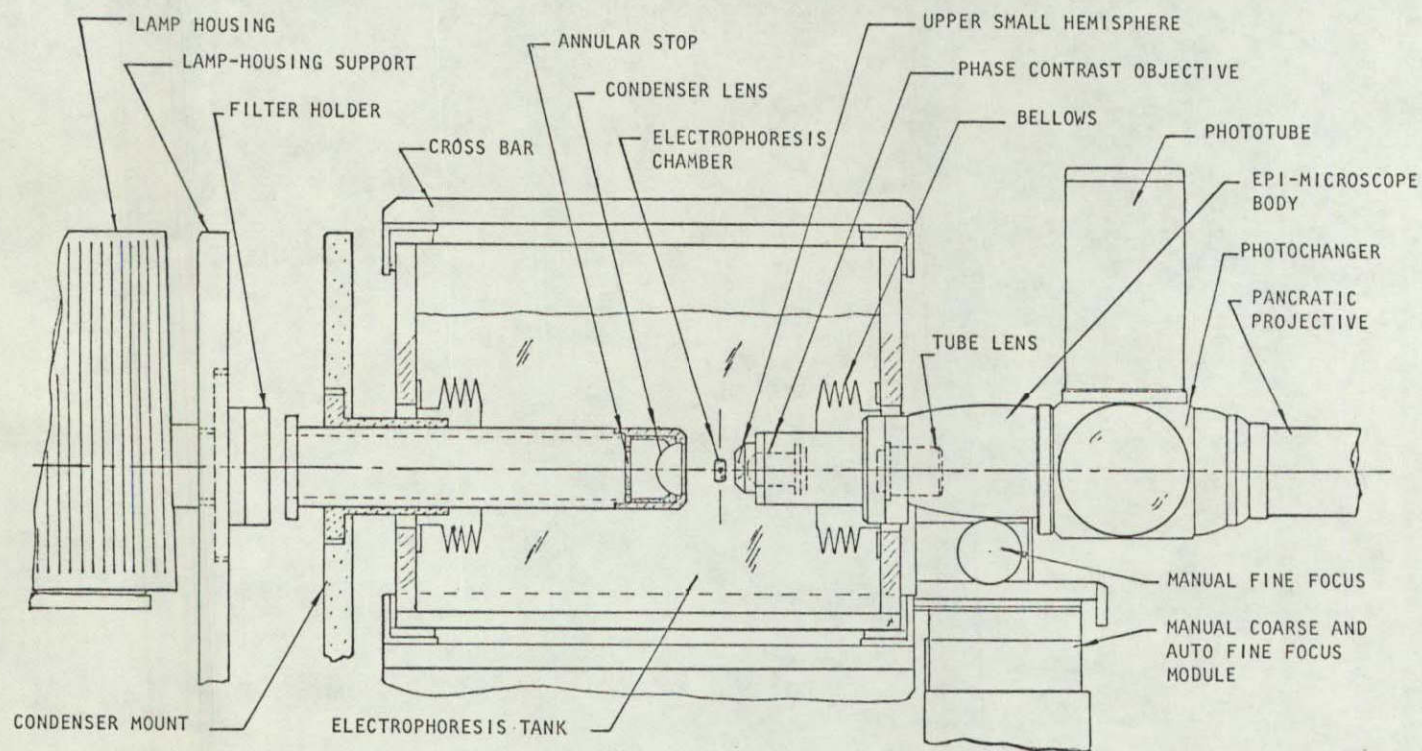


Fig. 14. Schematic of electrophoresis assembly--tank and optical components.

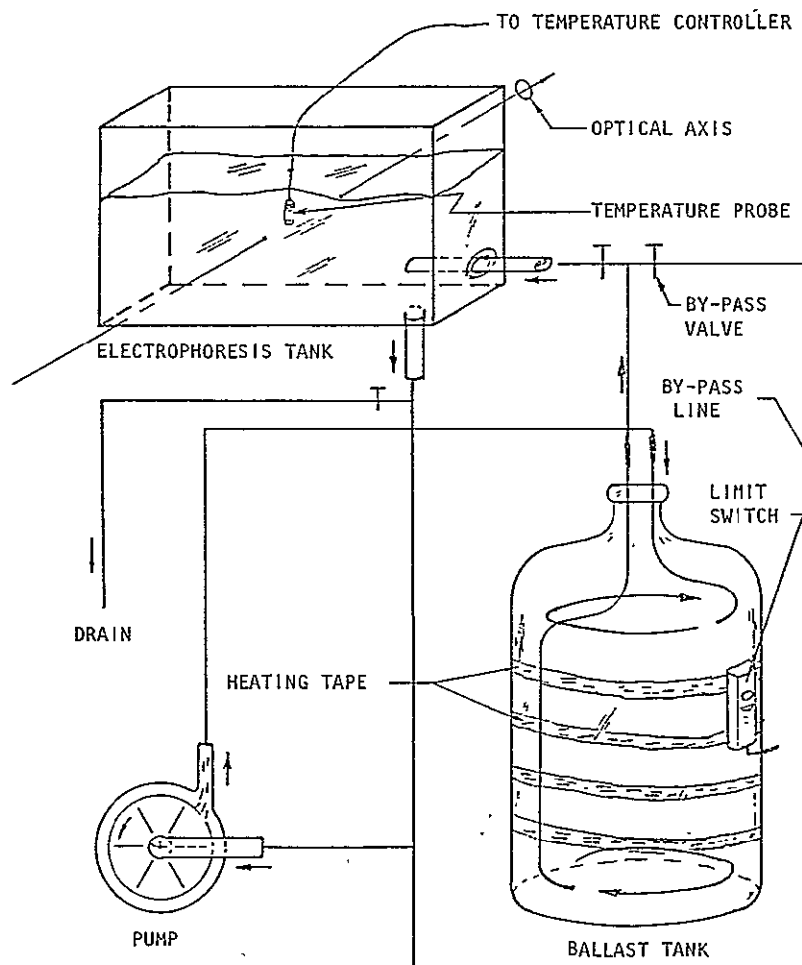


Fig. 15. Water circulation and temperature control system schematic.

Epi-microscope Body (6). This unit is the basic support unit for the microscope components including the objective and the photochanger to which the rest of the optics are attached. The epi-microscope body has a fine focus control that permits obtaining an initial best focus manually. The unit is supported by a rack and pinion focus mechanism that is mounted to an optical bench carrier.

Photochanger (5). The purpose of the photochanger is to allow the beam from the epi-microscope body to be directed into more than one tube either individually or simultaneously. For this instrument the horizontal port of the photochanger lets the beam from the epi-microscope body continue undeviated to the pancratic projective while the vertical port directs the same beam deviated by 90° to the phototube. In the extreme positions the prism cluster directs all of the light to one or the other of the two ports. In the intermediate position 60% continues undeviated and 40% is deviated 90° .

Pancratic Projective with Tube Lens Insert (4). This unit replaces the normal eyepiece tube and eyepiece. It is a zoom projection lens providing 1 to 3.2X magnification of the beam, which strikes the vidicon of the TV camera after leaving the unit.

Phototube (7). The phototube is similar to a monocular eyepiece tube but has a sliding tube extension that allows its length to be varied by 25 mm to focus the eyepiece into the camera's entrance pupil. Its upper extremity has a mounting flange for a special camera tube, which is a Basic Body I for this instrument.

Basic Body I with Focusing Ocular (8). This unit has a special eyepiece that is parfocal with the camera film plane. A reticle in the eyepiece allows exact centering and framing of the area to be photographed. Any

photographic camera (35 mm, 5 x 5 Polaroid, etc.) having the correct mounting flange can be used with this unit.

8X Kp1 Ocular. The ocular is placed in the top of the phototube (7) for use with the Basic Body I or it can be used with the eye alone for focusing the microscope.

12.5X Centering Telescope. The centering telescope is used in place of the 8X Kp1 eyepiece to observe simultaneously the phase ring in the objective and the annular stop in the condenser so that they can be aligned and centered relative to one another. It should be checked periodically to maintain phase contrast alignment.

TV Camera and Connecting Tubes (2). The TV camera is aligned with the optical axis of the system with light from the projective lens falling directly on the vidicon. Aluminum tubes are used between the projective lens and the TV camera to prevent stray light from striking the vidicon.

Lamp Housing and Filters (11). A 12-V 100-W quartz-halogen lamp is used as the source of illumination for the system. The lamp housing provides for focusing and centering the lamp as well as focus and two axes of tilt for the spherical mirror located behind it. Heat absorbing and reflecting filters are installed in the lamp housing to prevent the infrared portion of the spectrum from reaching the electrophoresis chamber.

Basic Design Mechanical Assembly

Structural Support (1). The structural support of this instrument is obtained from the use of Klinger X-95 beam members that have a 95-mm square profile. The X-95 configuration for this unit has the shape of a dagger. Vertical plates are added to the ends of the short crossbar for support of

a horizontal plate on which the Rank electrophoresis tank rests. The long arm of the dagger supports optical bench carriers on which are placed the microscope components (excluding the condenser), the autofocus motor, and the TV camera. The short arm at the opposite end supports the microscope condenser and the lamp housing on carriers.

Electrophoresis Tank Frame (10). The Rank tank is constructed from brass angle plate to support the electrophoresis chamber and the dial gauges. One of these is used to determine the depth of the electrophoresis chamber. The other is used to indicate the location of the microscope relative to the chamber.

Autofocus Unit (9). The autofocus unit consists of a stepping motor that is geared 1:2 with a timing belt to the fine focus knob of a focusing module on which the microscope is mounted. Movement of the fine focus knob by the stepping motor causes the microscope to move along its axis. Actuation of the stepping motor itself is done through the stepper motor driver. This in turn receives its signals from computer processed video information. The focus module also has a rack and pinion section for coarse focus. Both the coarse and fine focus dovetail slides were adjusted to give a minimum of side play and still provide smooth linear motion.

Condenser Mount (Fig. 12). The condenser can be adjusted for focus, centering, and two components of tilt so that it can be aligned and centered relative to the phase contrast objective. This mechanism is mounted to one of the X-95 carriers.

Lamp Housing Mount (Fig. 12). The lamp housing is mounted to a vertical plate that allows it to be centered relative to the condenser. The unit can be moved along the optical axis by an X-95 carrier. Filter holders

were threaded concentric to the lamp housing output and to the lamp housing mounting flange.

Vibration Isolation (not numbered in photograph, but shown clearly). To isolate the AAEA unit a concrete slab measuring 5 x 28 x 58 in. and weighing in excess of 600 lb was floated on a 30 x 60 in. tabletop using seven small thick-wall inner tubes. The table top was constructed of 1.5 in. thick wood (actual thickness) supported by 4 x 8 in. legs to carry the weight of the concrete with negligible sag. This greatly reduced the problem of vibrations in the AAEA unit induced by the building.

Basic Design of Water Circulation and Temperature Control System

The circulation system consists of the Rank electrophoresis water tank, a water ballast tank, and a centrifugal pump (Fig. 13).

The electrophoresis tank holds approximately 1.75 gallons of distilled water when it is filled to the level of the top of the horizontal electrophoretic chamber. The input and output are 3/8 in. inside diameter tubing over the side of the tank. A drain is located in one corner of the bottom of the tank.

The ballast is a 5-gallon distilled water bottle fitted with a three-hole rubber stopper. The stopper, which is clamped in place so that it cannot slip out, makes the ballast a sealed unit. Two holes in the stopper are for 3/8 in. stainless steel tubing--one for the input and the other for the output. The third hole is for either a temperature probe or a plug that can be removed to adjust the airspace at the top of the tank. The end of the input tube is located 75 mm below the surface of the water and the output is a similar distance above the bottom. Both tubes are bent so that they

are parallel to the inside diameter of the glass container for about 75 mm and about 40 mm from the inside wall. One tube faces the other, i.e., the water after leaving one spirals downward in the bath and enters the other.

Water normally runs from the pump to the ballast tank and then to the electrophoresis tank, all in series. The circulation, however, can be modified with hose clamps so that more circulates through the ballast. With the electrophoresis tank drained for cleaning, the hose to this tank can be closed and the clamp to the tank by-pass can be opened allowing the water to continue to circulate in the ballast tank.

The pump consists of a centrifugal rotor capable of pumping 215 gallons per hour at 3 ft of head magnetically coupled to a 5000 rpm, 1/20 H.P. universal motor with variable speed control.

The water temperature control system consists of a thermistor type sensor heated in the electrophoresis tank in close proximity to the electrophoresis chamber, a proportional temperature controller, and two series-connected glass tape heating tapes. The heating tape is wrapped around the ballast bottle in a spiral. Two 0.015 in. aluminum plates were formed to fit the outside circumference of the bottle to more uniformly distribute the heat from the tapes over the glass. These plates occupy half the bottle circumference each and are placed between the heating tapes and the glass bottle.

A limit switch is located on the outside of the ballast tank. This will turn the temperature controller off at 50°C should the tank temperature increase excessively.

III. PLANS FOR SECOND CONTRACTUAL PERIOD

The tasks for the second contractual year, which presently ends contractually on 15 February 1977, are to

- (1) Adapt and transfer existing data acquisition software to the dedicated (Eclipse S/130) computer.
- (2) Commence measurement of cell populations to establish the various performance parameters of the AAEA.
- (3) Design and test analytical and data handling software.

Originally, we proposed a task to commence statistical analysis of apparatus performance in measuring cell populations to identify significant sources of measurement errors. This assumed that the contractual period would end on 16 May 1978 rather than on 15 February 1977 and that funds would be available for essentially a 12-month rather than the present second contractual period of 9 months.

A. Data Processing Hardware and Software

We will commence red blood and some lymphocyte cell scanning and tracking tests using the 11/03 computer. Upon delivery of the Eclipse S/130 computer, we will modify the video compressor interface for the Eclipse, followed by complete system integration. We will then start scanning and tracking red blood cells, which have a well known mobility and will serve as a calibration for the AAEA. If time permits we will commence tracking lymphocytes prior to meeting the design objective of 500 cells per 10 min.

In the software area we will adapt and transfer existing data acquisition software from the PDP 11/03 computer to the dedicated Eclipse S/130

computer, which has its own operating system (RDOS). This includes applicable subprograms tested on the PDP 11/45 for data analysis. Programs will be modified as required in the light of AAEA test experience. In addition, we will design, develop, and test analytical programs for the Eclipse.

We see no major problem areas in software development for data acquisition and command and control. For data analysis, program development, the potential problem is that there may not be adequate time to commence that effort and make any progress by the present contract termination date of 15 February 1978.

B. Optomechanical

The major efforts remaining are to provide a vertical electrophoresis chamber capability, to provide a filter in the water circulation system to reduce turbidity, and to reduce vibration from whatever cause. During the test and evaluation phase of the AAEA, some unanticipated problems will, no doubt, emerge. Presently unanticipated optomechanical problems will be corrected as they occur.

IV. POTENTIAL PROBLEM AREAS

During our first good look at red blood cells under electrophoresis, we observed that the autofocusing action did not fulfill our design requirements in that tracking a moving target will evidently require additional logic. Autofocus devices, as described in the literature, have not been applied to moving targets. Motion of the target could introduce changes to the merit function. The additional logic should therefore safeguard the system from this eventuality.

In the laminar flow region of the capillary, the motion of the cells is slow and smooth enough so that automatic focusing is not required to the extent that the AFU is presently designed. However, inasmuch as we desire to track as many cells as possible, it is desirable to use a computer-controlled focusing system to find additional cells at other depths within the electrophoresis chamber. In this case, the computer will need to know where the focusing mechanism is at all times in order to stay within the laminar region or to correct the cell mobilities for boundary effects near the chamber walls.

We presently propose to feed the analog focus signal to the computer (consisting of unipolar high frequency information), which will then drive the stepper motor to a new position. If the cell does start to move out of focus during tracking, the focus algorithm in the computer can perform the necessary correction by driving the stepper motor in the proper direction and amount. Using this approach, we believe that we can track more cells in low concentrations than we could previously. It also means that

the stepper motor will be driven much less, thus further reducing vibration from that source.

V. CONCLUSIONS AND RECOMMENDATIONS

It can be concluded that we have made good progress to date in the design of a feasible automated analytical electrophoresis apparatus. This performance has been within contractual cost and time goals and as proposed.

It is strongly recommended that this program be continued during the period 16 February to 16 May 1978 to allow AAEA testing and evaluation using live cells to determine and measure its operating parameters and to make necessary (and inevitable) minor changes, in order to have the AAEA ready for clinical testing and evaluation during the third, or subsequent, period as originally proposed. If this is not done, a potentially valuable apparatus will not have its operating parameters measured, and thus defined, nor will it be ready for clinical test and evaluation.

We strongly recommend also that a third or subsequent period be funded for clinical testing and evaluation. During this time, data analysis programs must be further developed, tested, debugged, and applied in order that the specified design objectives can be attained. Experience will, no doubt, suggest AAEA modifications to improve its performance such as improvements in the autofocus system electronics, addition of a printer terminal for processing of hard copy, and the capability of access by two system operators. In this latter case, one user could work on program development or data analysis (in background), while the other user could be operating the computer to obtain data (in foreground). The computer's RDOS operating system permits such dual, simultaneous operation. Also, it would be desirable to acquire and install a camera to the microscope for photographing

the objects in the field of view. In addition, experiments in distinguishing cells with and without fluorescent labels should be undertaken.

VI. EQUIPMENT AND ASSOCIATED COMPONENT HARDWARE COSTS

Estimated contract funds expended on major and associated hardware to 31 August 1977, for AAEA fabrication and assembly, are summarized in the following categories.

A. Electronics and data processing

1. TV cameras and monitors	\$ 1,197	
2. I/O for PDP 11/03 and cabling	720	
3. Power supply	82	
4. TV waveform monitor	1,331	
5. Colorado Video, video compressor	(3,000) ¹	
6. Video processor	(1,000) ¹	
7. Stepper motor and stepper driver	1,231	
8. Digital/analog converter	218	
Total equipment	4,779	
9. Electronic components for fabrication of units and of interfaces	903	
Subtotal electronics and data processing		\$ 5,682

B. Optical and mechanical

1. Rank electrophoresis microscope assembly	269	
2. Carl Zeiss microscope system and associated optical components	5,313	
Total optical equipment	5,582	
3. Supporting structure components; optics, rail, carriages, adapters, etc.	885	
Total optomechanical equipment	6,467	
4. Mechanical and miscellaneous optical components for fabrication and assembly of supporting structure and water circulation and temperature control system	834	
Subtotal optomechanical		\$ 7,301

C. Direct services

Instrument shop labor for fabrication	3,768	
Electronics shop labor, same	404	
Design draftsman	710	
Factory maintenance, 12 mo. PDP 11/03	1,094	
Total		\$ 5,976

Estimated total direct costs for fabrication and assembly of AAEA	\$18,862
Major components on order but not yet received nor paid for by contract: Eclipse S/130 computer and peripherals	<u>30,649</u>
Estimated total direct apparatus cost to 8/31/77	<u>\$49,511</u>

Note 1: It should be noted that the above costs do not include the indirect costs of program engineering, management, and administrative support labor and other administrative and operating costs. It does not include the cost of the video compressor, which was donated to the University of Arizona by the Colorado Video Corporation and which will become government property on delivery of the apparatus. The video processor employed was also at no cost, as it was government surplus equipment acquired by the University. It too will become government property upon equipment delivery.

The foregoing can be summarized also as follows:

Major electronic and data processing optical equipment on hand	\$ 4,779	
Same, data processing equipment on order	<u>30,649</u>	
Subtotal electronic and DP equipment		\$ 35,428
Major optomechanical equipment on hand	6,467	
Subtotal special optomechanical equipment		<u>6,467</u>
Subtotal special equipment		<u>\$ 41,895</u>
Electronic components for fabrication, interfacing, assembly, and test of electronics and data processing equipments		903
Optomechanical components for fabrication, assembly, and test of optomechanical and water circulation and temperature control systems		<u>834</u>
Total miscellaneous materials, supplies, and components		<u>1,737</u>
Total direct shop labor for fabrication of optomechanical assemblies		3,768
Same for fabrication and assembly of electronic equipments		<u>404</u>
Total direct shop labor		<u>\$ 4,172</u>
Services; drafting and computer factory maintenance contract		<u>\$ 1,804</u>
Total estimated direct apparatus cost		<u>\$ 49,511</u>

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